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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. B.S. Heming

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# THE EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON EMBRYOGENESIS OF *MAMESTRA CONFIGURATA* (WLK) (LEPIDOPTERA: NOCTUIDAE)

BY

©

MICHAEL P. JONES

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF THE MASTER OF SCIENCE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Temperature and Relative Humidity on Embryogenesis of Mamestra configurata (Wlk) (Lepidoptera: Noctuidae) submitted by Michael Paul Jones in partial fulfilment of the requirements for the degree of Master of Science.

B. S. Heming
Supervisor

Dated October 7, 1976

## ABSTRACT

The effects of various combinations of temperature and relative humidity on embryogenesis in *Mamestra configurata* (Walker) were investigated and features of the egg were described. Temperature thresholds were determined for some stages of embryogenesis (developmental-hatching, (8.5° C), hatching, (5° C), developmental (between 0 and 2° C) and the high temperature developmental-hatching threshold, (30° C)). Temperature and rate of development curves were derived using three different relative humidities (0, 60 and 98%) and a range of temperatures (8.5 - 30° C). Length of exposure to 35° C and 5° C required to produce mortality of 50% and 95% was determined for eggs of various ages. The ages of eggs exposed to 35° C did not appear to influence mortality. However, age was very important in eggs exposed to 5° C. The older the eggs, the longer the exposure required to produce 50% and 95% mortality.

The effect of daily exposure to 35° C and 5° C was studied for eggs of various ages. Older eggs could tolerate longer daily exposure to 35° C without high mortality than could younger eggs. However, when the total time of exposure was determined, there appeared to be no difference in tolerance between older and younger eggs. Daily exposure to 5° C had little effect on mortality but lengthened the period of development.

Results of this study show that relative humidity can influence rate of development. The developmental rate and temperature curves for *M. configurata* appears to be J-shaped. Practical application of these curves will result in greater accuracy in larval surveys and in more efficient control of this pest insect.

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I thank the staff of the Plant Industry Laboratory, Alberta Department of Agriculture for providing facilities where most research reported in this thesis was conducted.

Thanks are due also to the staff of the Crop Entomology Section, Lethbridge Research Station, Agriculture Canada, for their help and for letting me use their facilities during the initial part of this research.

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Finally, a very special thanks to the late Dr. Brian Hocking, former Chairman, Department of Entomology, University of Alberta for supporting me financially on AART Grant No. 55-28158 - "Biology and Behaviour of Noctuidae".

## AUTOBIOGRAPHICAL SKETCH

I was born in Ottawa, Ontario in 1949 and received all my education in Alberta. I developed a strong interest in biology in senior high school and continued my interest in the University of Lethbridge.

During my third year of university, I worked as a research assistant for the Lethbridge Research Station, Agriculture Canada, Crop Entomology Section, where I became fascinated with insects. After my second summer there I became committed to studing entomology at the graduate level.

During my summers at Lethbridge, my main interest was in crop entomology. This, more than any other reason, was why I chose this insect and this research project.

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# INTRODUCTION

The Bertha armyworm, *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae), has a known geographic range extending from Mexico City, Mexico (King, 1928) to Keg River, Alberta (Philip, 1973). Within this range, it is of economic importance only in Western Canada and in the state of Washington.

In Western Canada, the importance of M. *configurata* has increased lately due, in part, to the recent great increase in rape (*Brassica campestris*) acreage. After Berthas first serious outbreak in 1971, the Alberta Department of Agriculture instituted a series of four annual surveys to determine, first, the areas where densities of M. *configurata* were high and where the potential for economic damage great, and second, to follow the changing distribution of M. *configurata* in Alberta each year.

A fall pupal survey is conducted in areas where outbreaks occurred that year, and its purpose is to estimate the initial size of overwintering populations. A second pupal survey is conducted in the spring to ascertain the winter mortality of overwintering populations. A third survey, conducted from mid-May until the end of September, involves the use of black light traps to monitor adult emergence and abundance. A final egg and larval survey, is conducted in most rape-growing areas of the province, usually in July. Based on the results of these surveys, a series of maps are prepared which indicate areas of potential economic damage for that crop year. The most important survey is the final one because this confirms the presence of the damaging stage in the fields.

The principal drawback of the final survey is its timing. For it to be effective, it should be conducted at that time when the majority of eggs have hatched but before the larvae have reached a size where they are causing economic damage. If the survey is conducted too early, before the majority of the population has hatched, the population estimate resulting may be too low. If left until too late, it may be impossible to devise and implement control measures before considerable damage has occurred.

There were three principal objectives in undertaking this study: (1) to remove some of the guess work involved in timing the egg and larval survey, by developing temperature curves for embryogenesis which could aid in predicting probable hatching time of the eggs in the field, (2) to determine the effects on development and viability of the eggs of exposure to various periods of unfavorable temperature, and, (3) to determine the role of relative humidity on embryonic development in this insect.

Most laboratory research was conducted in the facilities of the Alberta Department of Agriculture, Plant Industry Division, O.S. Longman Building, Edmonton.

# 2. LITERATURE REVIEW

## 2.1 *Mamestra configurata* (Walker)

*M. configurata* (*Barathra configurata*) first appeared in the economic entomological literature in 1928 in a paper by King (1928). That paper dealt with the external morphology of its various lifestages and with some aspects of its life history including its geographic range and host plant records (rape was not included). He also gave descriptions of larval damage on various crop plants. His larval and pupal descriptions are still helpful in separating specimens of *M. configurata* from those of other noctuids causing damage to rape.

Embryogenesis of *M. configurata*, from pre-fertilization to eclosion, was described by Rempel (1951). He also reported on some of the ovipositional habits of this moth and enlarged upon King's (1928) observations of its egg. The study included a brief description of the external structure of the egg. One of the more important observations he made, which had important implications for my own research, was that fertilization, at room temperature (20° - 21° C), occurred in the second half hour after oviposition.

The serious outbreaks of 1971 and 1972 on rape have resulted in the implementation of numerous studies, the results of which are now just being published.

The male and female pheromones of *M. configurata* have been studied

by Clearwater (1975) and Struble, et al, (1975) respectively.

Various methods of conducting population surveys on this insect have been devised (Putnam, 1972; Dixon and Philip, 1973; and Swailes, et al, 1975).

The effects of various insecticides on larvae in the field have been studied by Jacobson (1972). Laboratory studies on the toxicity of insecticides to all lifestages were conducted by Harris and Turnbull (1975). The effect of various predators on larval mortality has also been studied (Tamaki and Weeks, 1972).

## 2.2 Temperature thresholds for embryogenesis

Study of the effects of temperature on organisms began when Reamur (1735, see Belehradek, 1930) recognised that a relationship exists between temperature and the activity of an animal. Since then, these relationships have been examined by numerous authors and reviewed by Crozier (1926), Belehradek (1930), Uvarov (1931), Janisch (1932), Howe (1967) and several others. The results of these studies led to the development of the concept of various temperature thresholds for development stages.

There are two principal types of temperature threshold; low temperature thresholds, involving temperatures too low for certain developmental processes to be completed, and high temperature thresholds, which consider temperatures too high for normal development. Determination of these thresholds, in embryogenesis, has been made primarily

in the first type, probably because of the larger temperature coefficients existing between the various thresholds at lower temperatures.

Presently, four low temperature thresholds are recognized as affecting embryogensis. Peairs (1927) defined the develomental threshold as "the temperature at which, on the descending scale, development ceases, and at which, on the ascending scale, development is initiated". Johnson (1940) introduced the hatching threshold and the developmental-hatching threshold which are, respectively, the lowest temperature at which hatching of a fully developed larva can occur, and the lowest temperature at which complete development from fertilization to eclosion can occur. The hatching-survival threshold was suggested by Hodson and Al Rawy (1956) and they chose Allee, et al's, (1949) definition of the "lowest temperature at which a given stage in the life history can be carried through to completion" to define it.

The only high temperature threshold recognized, for embryogenesis, is the high temperature equivalent of the developmental-hatching threshold. It has been suggested that there exists a high temperature threshold equivalent to the developmental threshold. However, Uvarov (1931) questioned whether this temperature is distinct from the upper lethal temperature. He further stated that if there was an appreciable difference in temperature between the upper lethal point and the upper developmental threshold, then there should be a quiescent stage similar to the quiescence observed at temperatures below the developmental threshold but above the lower lethal point. This stage, referred to as

heat stupor, has been recognized in relation to activity but, as yet, has not been described for development. Theoretically, this stage could exist, although the range between it and the upper lethal temperature may be so restricted that discovery will be of limited practical value.

The concept of the developmental threshold of Peairs (1927), has been given numerous names by various authors in attempts to convey with greater clarity the process that occurs at that particular temperature. It has been called "the critical point", "physiological zero" and "the minimum effective temperature" (Uvarov, 1931). Some more recent authors (e.g. Lin, et al, 1954), have chosen to ignore the original definition of this threshold and have used instead Johnson's (1940) developmental-hatching threshold definition as if it were synonymous. The developmental threshold is seldom determined analytically, but is generally arrived at by extrapolation from temperature and rate of development curves. This method assumes that the effects of temperature on the length of time of embryogenesis can best be represented, mathematically, by a hyperbolic function (Sanderson and Peairs, 1914). This function, when transformed into its reciprocal, produces a straight line. This line is often called the "velocity line", and represents the effects of temperature on rate of development. When plotted on a graph, the point where the velocity line intercepts the temperature axis is the hypothetical zero of the hyperbola or the developmental threshold. This method is used extensively to derive the developmental threshold; for example it is 11.5°C for the Corn Earworm (*Heliothis zea* [Boddie]

Lepidoptera:Noctuidae) (Luckmann, 1963) and 11° C for the European Corn Borer (*Ostrinia nubilalis* [Hübner] Lepidoptera:Pyralidae) (Matteson and Decker, 1965).

The difficulty in using a straight line to describe rate of development was first pointed out by Krogh (1914) and later by Shelford (1927) and Peairs (1927). Peairs (1927) noted that the development rate deviated from a straight line near the extremes of the temperature range. Thus, thresholds determined using this method were higher than the actual threshold.

Johnson (1940) emphasized the need for determining thresholds emperically and the necessity of recognizing different thresholds for various stages of embryogenesis. He suggested that because there are distinct stages in embryogenesis that there should also be equally distinct temperature thresholds for development of these stages. He suggested that both hatching and developmental-hatching thresholds be used. However, with the exception of a study on the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera:Lygaeidae) which showed it to hatch at a temperature 2° C lower than its developmental-hatching threshold if 90 per cent of the previous embryonic development occurred at 20° C (Lin, et al, 1954), most researchers have chosen to disregard both the hatching and the developmental-hatching thresholds.

The hatching survival threshold used by Hodson and Al Rawy (1956) has also been ignored by recent workers, probably because of the extensive amount of time required for rearing immatures through to adulthood.

The upper temperature developmental limit can be considered to be the upper equivalent of the developmental-hatching threshold or the highest temperature at which complete development and eclosion can occur. This upper threshold has been described in representative species of many orders of insects, e.g. *Onychiurus furciferus* (Borner) (Collembola: Onychiuridae) (Choudhuri, 1960), *Geocoris atricolour* Montd. (Hemiptera: Lygaeidae) (Dunbar and Bacon, 1972), *Phormia regina* Meig. (Diptera: Calliphoridae) (Melvin, 1934), *Epilachna corrupta* Mulsant (Coleoptera: Coccinellidae) (Pyenson and Sweetman, 1931) and *Telea polyphemus* Cramer (Lepidoptera:Saturniidae) (Ludwick and Anderson, 1942). Bursell (1974) listed the upper limit for various insect species, the highest, 40° C, being recorded for *Ptinus tectus* Boield (Coleoptera:Ptinidae), the lowest, 28° C, for *Tribolium confusum* (Herbst) (Coleoptera:Tenebrionidae).

## 2.3 The effects of temperature and relative humidity on embryogenesis

Research conducted on the effects of temperature on insect embryogenesis can be broadly classed into two groups. The first treat the effects of constant temperatures (often applied at different relative humidities); the second, the effects of alternating or varying temperatures on embryonic development. This subject has been reviewed by Sanderson (1910), Uvarov (1931), Janisch (1932), Bursell (1974) and (for embryogenesis only) Howe (1967). The effects of relative humidity have been reviewed by Buxton (1932) and Ludwick (1945).

One of the principal reasons that the effects of constant temperature on insect development is studied, is the hope that the resulting developmental curves can be used to predict insect development in the field. Numerous attempts have been made to derive a general mathematical function or equation that describes the relationship between temperature and development in insects. These attempts have been reviewed by Crozier (1926), Uvarov (1931), Janisch (1932), Davidson (1944), and Howe (1967). The more widely used of these concepts are those of: day-degrees; thermal summation and summation of development units.

Improper use of these concepts often leads to grossly inaccurate results. This occurs when fluctuating temperatures are assessed in terms of a simple average rather than weighed according to the effects of each individual temperature on development (Bursell, 1974). The inaccuracy of these concepts arises from the assumption that the best method to represent mathematically the effect of temperature on rate of development, is through the use of a straight line. Near the extremes of the temperature range for a species, the rate of development deviates from a simple linear relationship. At the lower extreme, the rate of development declines more slowly; at the upper extreme, more rapidly than would be expected for a strictly linear relationship. The resulting temperature and rate of development curve is thus closer to being sigmoidal than linear. However, when the range between the maximum rate of development and the upper lethal limit is slight the resulting curve is J-shaped (Howe, 1967).

Relative humidity can apparently have the following effects on insect embryogenesis: (1) It can have no effect, (2) can cause changes in development time, (3) can influence mortality rate and (4) can influence developmental thresholds. These effects and others on invertibrates have been reviewed by Buxton (1932) and Ludwick (1945).

The development of many stored product and household pests does not appear to be affected by humidity. In three species of Tenebrionidae (*Tribolium castaneum* [Herbst]. *T. confusum* [Herbst] and *T. madens* [Charp] [Coleoptera:Tenebrionidae]) no measurable effects on development were recorded for various relative humidities in combination with various constant temperatures (Howe, 1956, 1960 and 1962). Similar results were reported for the webbing clothes moth, *Tineola biselliella* Hum. (Lepidoptera:Pyralidae) (Griswold and Crowell, 1936), the Mediterranean flour moth, *Ephestia kuhniella* Zeller (Lepidoptera:Pyralidae) (Ahmed, 1936) and for the firebrat, *Thermobia domestica* (Packard) (Thysanura:Lepismatidae) (Sweetman, 1938).

For many insects, the rate of embryogenesis declines as relative humidity declines. At a constant temperature of 28° C, eggs of both *Plodia interpunctella* (Hübner) (Lepidoptera:Phycitidae) and *Crambus teterrellus* (Zincken) (Lepidoptera:Pyralidae) showed a definite delay at low humidities (Morrison and Crawford, 1970; Morrison, et al, 1972). A delay in embryogenesis occurred at low humidities at all temperatures tested in *Lasioderma serricorne* (Fabr.) (Coleoptera:Anobiidae) (Powell, 1931) and *Epilachiia corrupta* (Pyenson and Sweetman, 1931). This delay can be quite striking. For example, the development of *Sitona lineata* L. (Coleoptera:Curculionidae) at 20° C required 10.3 fewer days at app-

roximately 100% RH than at 62% RH (Andersen, in Buxton, 1931). The reasons postulated for this delay are: the larvae may be weakened by water loss and thus take longer to hatch (Pyenson and Sweetman, 1931) and/or the chorion, because of desiccation, hardens, making escape of the insect more difficult (Pyenson and Sweetman, 1931; Howe and Burgess, 1953).

However, not all insects show these results of either no effect ora delayin development due to low humidity. For example, the rate of embryogenesis for four saturniid moths (*Platysamia cecropia* L, *Telea polyphemus*, *Samia walkeri* Felder and Felder and *Callosamia promethea* Drury) were effected inconsistently and at optimum temperatures demonstrated no responses on exposure to different relative humidities (Ludwick and Anderson, 1942). The bean weevil, *Acanthoscelides (Bruchus) obtectus* (Say) (Coleoptera:Bruchidae) reacted in the opposite way to relative humidity, requiring six days to develop in saturated air at 27° C and only four days at the same temperature and 24% RH (Headlee, 1921).

The exact mechanism or mechanisms by which relative humidity affects development and mortality are unknown. Development of *P. cecropia* occurred over a much wider humidity range than did hatching (Ludwick and Anderson, 1942). They found that for eclosion, humidities of 56% and 76% were more favourable than either dry or saturated air. The cause of the adverse effects of saturated air on embryogenesis is unknown. However, saturated air appears to aid fungus or mold growth on some insect

eggs which may cause the death of the latter (Ludwick and Anderson, 1942; Lin et al, 1954). The blow fly, *Lucilia sericata* Meig. (Diptera: Calliphoridae) showed prolonged development, leading to increased mortality, when exposed to low humidity (Evans, 1934). The cause of this mortality was possibly due to one or a combination of three causes: (1) the larva is weakened by water loss and is unable to hatch, (2) the chorion may harden, making it impossible for the larva to hatch (experimental evidence demonstrates that this occurs in eggs of the walking stick, *Diapheromera femirata* (Say) (Orthoptera:Phasmidae) (Severin and Severin, 1910), (3) the embryo may die before it is ready to hatch (this occurs in embryos of *Haematopinus asini* (Linne)=(*H. marocephalus* Burmeister) (Anoplura:Haematopinidae) (Bacot and Linzell, 1919).

The effects of humidity in modifying developmental thresholds have been little investigated. Low humidities or very high humidities at lower threshold temperatures, appear to be unfavourable to eggs of the milkweed bug, *O. fasciatus* where they have the effect of raising the threshold temperature 1 or 2° C (Lin, et al, 1954). At temperatures above optimum, the eggs of *P. cecropia*, *T. polyphemus* *S. walkeri*, and *C. promethea* had a lower mortality rate at high humidities than low humidities (Ludwick and Anderson, 1942). Similar results are reported for eggs of *H. zea*, *H. virescens* (F.) (Lepidoptera: Noctuidae), *Trichoplusia ni* (Hübner) (Lepidoptera:Noctuidae), *Pectinophora gossypiella* (Saunders) (Lepidoptera:Gelechiidae), and *Estigmene*

*acrea* (Drury) (Lepidoptera:Arctiidae) which, when exposed daily to high temperatures showed a higher percentage hatch at high humidities (95% RH) than at lower humidities (Fye and Surber, 1971). At temperatures below optimum, eggs of *Rhodnius prolixus* Stål, (Hemiptera: Reduviidae) hatch only at humidities considered to be optimal (Clark, 1935). This general trend was also reported for eggs of *E. corrupta* (Pyenson and Sweetman) and *P. cecropia* (Ludwick and Anderson, 1942).

Research on the effects of alternating temperature on insect embryogenesis is of the following types: (1) a low temperature is alternated with a medial temperature (medial temperatures are a range of temperatures for an insect species, where the rate of development most closely approaches a linear relationship with temperature); (2) a high is alternated with a medial, (3) two medial temperatures are alternated with each other, and (4) a template based on field conditions is used to mimic natural conditions.

Temperature alteration can be accomplished by use of a number of different methods. Eggs of the Japanese beetle, *Popillia japonica* Newman (Coleoptera:Scarabaeidae) were exposed to one temperature for various lengths of time and then transferred to a second temperature for the remainder of the experiment (Ludwick, 1930). Richards and Suanraksa, (1962) used multiple exposure to one temperature followed by exposure to another temperature. A template based on actual field conditions was used to study the effects of temperature on embryogenesis in *Dacus dorsalis* Hendel, *D. cucurbitae* Coy and *Ceratitis capitata*

(Wied) (Diptera:Tephritidae) (Messenger and Flitters, 1959). Regardless of the method used, alternating temperatures have been shown to have some effect on embryogenesis.

These effects have been reviewed by Uvarov (1931), Howe (1967), Wigglesworth (1965) and others. In general, such treatment can (1) accelerate development rate, (2) decrease development rate, (3) influence the developmental-hatching threshold, (4) influence mortality, (5) or have no effect.

When a low temperature, usually considered to be below the developmental threshold, alternates with a medial temperature, development of the insect appears to be accelerated when compared with its rate of development at a constant temperature equivalent to the mean of the alternating temperatures. Such accelerated development under alternating temperatures has been reported for the egg, larval and pupal stages of the codling moth, *Carpocapsa pomonella* L., (Lepidoptera: Olethreutidae) (Shelford, 1927) and the Japanese beetle, *P. japonica* (Ludwick, 1930); for the larval stage of the army cutworm (*Chorizagrotis auxiliaris* Grote) (Lepidoptera:Noctuidae) (Cook, 1927), for pupae of *Drosophilia melanogaster* Meigen (Diptera:Drosophilidae) (Ludwick and Cable, 1933) and *Spilosoma lubricipeda* (L.) (Lepidoptera:Arctiidae) (Baker, 1971) and for eggs of *Cimex lectularius* L. (Hemiptera:Cimicidae) (Johnson, 1940), *Eutettix tenellas* (Baker) (Homoptera:Cicadellidae) (Harries, 1943), *D. dorsalis*, *D. cucubitae*, *C. capitata* (Messenger and

Flitters, 1959) and *S. lubricipeda* (Baker, 1971). Several reasons for this apparent acceleration have been postulated: (1) the apparent acceleration is caused by some development actually occurring at the lower temperature (Ludwick and Cable, 1933), (2) acceleration of development arises from the fact that the temperature above the mean produces a relatively greater effect than the fluctuations below the mean, and (3) an acceleration in development is due to the temperature fluctuation itself (Johnson, 1940). The second cause would operate if the temperature and rate of development curve deviated from a linear relationship as it has been shown to do (Sanderson, 1910; Messenger and Flitters, 1958).

Alternating temperature experiments, in which a low temperature alternates with a medial temperature, do not always produce acceleration. Eggs of the milkweed bug, *Oncopeltez fasciatus*, show inconclusive results as acceleration of development occurred only occasionally (Lin, et al, 1954). Hodson and Al Rawy (1956), found that eggs of this bug showed no evidence of acceleration under alternating temperatures. While both larval and pupal stages of the European corn borer, *O. nubilalis*, showed some signs of developmental acceleration under alternating temperatures, the eggs showed no evidence of acceleration (Matteson and Decker, 1965). Eggs of the Japanese beetle, *P. japonica*, showed a retardation of development after exposure to a low temperature, (Ludwick, 1928). The seeming accelerating effect of low temperature may be misleading according to Richards and Suanraksa, (1962). They felt for *O. fasciatus*, that "the

retarding and debilitating effect of 17° C or lower is ameliorated by the relatively small percentage of time spent at a favourable temperature". They suggested that some phase in development cannot occur properly or at all at lower temperatures.

When a medial temperature is alternated with a temperature above the thermal optimum development of the insect appears to be retarded when compared with its development at the mean constant temperature. This general trend has been reported in: *P. japonica* (Ludwick, 1928), *D. melanogaster* (Ludwick and Cable, 1933), *D. dorsalis*, *D. cucurbitae* and *C. capitata* (Messenger and Flitters, 1959). One reason theorized for the apparent retarding effect of high temperatures is that due to the probable sigmoidal nature of the temperature and rate of development curve, the rate of development begins to decline rapidly at temperatures above the thermal optimum. Thus, the high temperature is not having as great an effect on the speed of development as would be supposed if the relationship was truly linear (Johnson, 1940).

A number of previously mentioned authors (e.g. Harries, 1943; Messenger and Flitters, 1958) noted that alternation between two medial temperatures has no apparent effect on rate of development. This result is not surprising as the relationship between temperature and development rate usually approaches a linear relationship within the medial temperature range.

Alternating temperatures can lower the constant temperature developmental-hatching threshold by several degrees. Development to eclosion of *Onco-*

*peltus fasciatus*, could occur under alternating temperatures even when the mean temperature was 1° C lower than the constant temperature threshold (Lin, et al, 1954). Similar results were reported for three species of fruit flies by Messenger and Flitters (1958). They found that complete embryogenesis could occur at mean temperatures 1° C lower than the constant temperature threshold. A higher percentage hatch of *O. fasciatus* occurred at alternating temperatures, averaging the constant temperature developmental-hatching threshold, than at the corresponding constant temperature (Lin, et al, 1954). They supposed that some process of development that cannot be completed at a lower temperature will become the limiting factor at a constant temperature, whereas at alternating temperatures this process can be completed at the higher temperature.

## 3. THE EGG

My preliminary reason for undertaking this part of the study was to examine the chorion for areas that might be permeable to water. In addition I wanted to provide an accurate description of the egg. I was also interested in determining whether any of my handling techniques were having adverse effects on egg viability. For a major portion of this study, the age of the eggs had to be known which led me to devise a number of new techniques and to modify others.

## 3.1 Egg structure

The main reason for describing the egg of M. *configurata* is to provide a means by which others can recognize it. Although both King (1928) and Rempel (1951) described some features of the egg, both missed some important features.

Eggs of M. *configurata* are generally deposited on the underside of the leaves of the host plant in a tight, single-layered cluster. The eggs are oriented with their anterior, micropylar ends pointing away from substrate (Rempel, 1951). The eggs are a yellowish white when first deposited, the yellowish colouration disappearing several hours later leaving the eggs an off-white. Approximately 24 hours later (at 20° C), a band of brown pigment appears around the equator of fertile eggs (Rempel, 1951). Small patches of similar pigment also develop in the micropylar area. Approximately eight hours prior to hatching, the egg turns jet-black as the larval head capsule becomes visible through the transparent chorion. Peterson (1964) referred to this as the black spot stage.

Features of the eggs' chorion were examined by scanning electron microscopy (SEM.)

### *3.1.1 MATERIAL AND METHODS*

The eggs used came from a laboratory culture of adults, reared from field-collected pupae, maintained at 20 ± 0.5° C and 60% RH. They were

less than six hours old when placed in the fixative (70% ethyl alcohol). They were left for 24 hours in this fixative and were then gradually dehydrated through a graded alcohol series. The eggs were sonicated for 30 to 40 seconds in absolute alcohol to remove extraneous material. The alcohol was replaced with fresh absolute alcohol and a solution of 85% amyl acetate was gradually introduced. Once all the alcohol was removed, the eggs were left in the amyl acetate for about two hours. They were then dried in a Denton DCP-1 Critical Point Dryer.

The dried eggs were positioned on Scotch® double-sided tape attached to metal stubs, coated with carbon and gold and examined with a Cambridge S4 stereoscan microscope. The internal structure of the micropyle area was also examined. The chorion was removed using a technique devised by Rempel (1951). In some eggs the vitelline membrane was removed using the technique of Salkeld (1973). After removal, the chorion was treated as above. All measurements given are the means of at least 25 measurements listed in Appendix I.

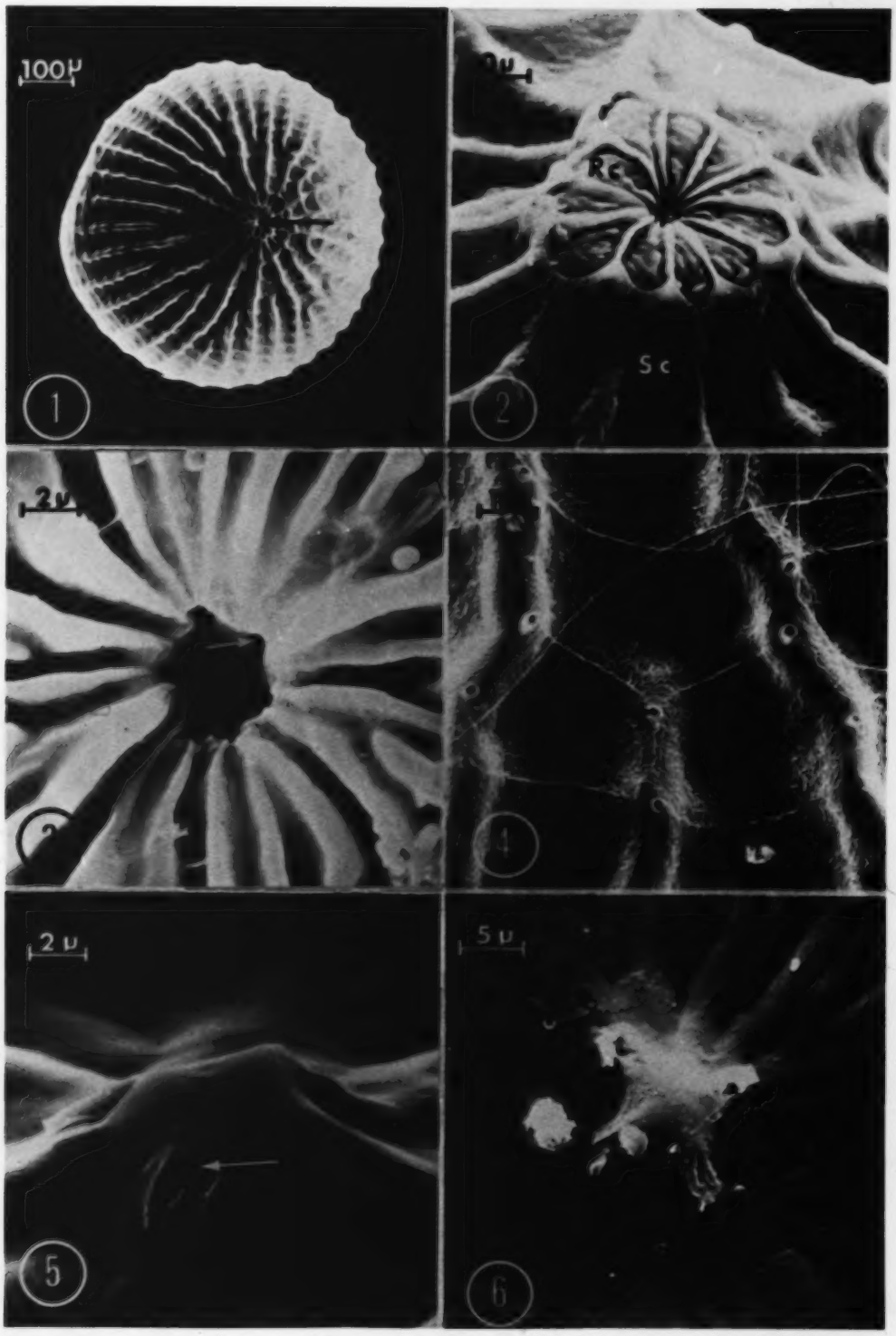
### 3.1.2 *OBSERVATIONS*

The egg of *M. configurata* is slightly wider than high, has an average diameter of 575.91 ± 27.12 μ, (based on 34 observations) and an average height of 409.68 ± 29.23 μ, (based on 25 observations). The number of primary cells (RC) ranges from 10 to 15 with a mean of 11.61 ± 1.28 (based on 91 observations).

The eggs (Figure 1) are spheroidal with their posterior surface slightly flattened. The chorion of each is covered with raised ridges or ribs except for its posterior surface. King (1928) recorded approximately 38 of these ribs while my own findings based on 41 observations suggest an average of 35.76 ± 2.06 (30-40). The ribs originate near the margin of the posterior surface and extend for various lengths cephalad, some ending at the level of the primary cells (Fig. 2, Rc), others approximately 4/5 the way up. Located between these vertical ribs are faint horizontal markings (Fig. 1). On the upper third of the egg where these markings meet a vertical rib there is a small opening, the aeropyle (arrow in Fig. 4) slightly less than 1 μ in diameter. In the center of the upper pole of the egg (arrow in Fig. 1) is a raised platform consisting of leaf-shaped primary cells (Fig. 2, Rc) surrounding the micropylar area. The sides of the platform are composed of secondary cells (Fig. 2, Sc). The micropylar area consists of a slight depression from which run three to six (mean 4.56 ± 0.7) (based on 50 observations) micropylar canals (see arrow Fig. 3).

The internal surface of the micropylar area consists of a sunken depression in the chorion (Fig. 5) from which run a varying number of tubes (the endomicropyles), each leading to an opening having a diameter of approximately 1 u (Fig. 5). These tubes are normally closed at their inner ends by the vitelline membrane (Fig. 6).

Figs 1 - 6 Scanning electron micrographs of the egg of *Mamestra configurata*. 1. Whole egg, arrow indicates micropyle area; 2. Micropyle area; Rc primary cells, Sc secondary cells; 3. CLose-up of micropyle area, arrow indicates opening to endomicropyle tube; 4. Ribs of chorion; arrow indicates an aeropyle; 5. Interior surface of the micropyle area with vitelline membrane removed; arrow indicates the opening of the endomicropyle; 6. Same, with the vitelline membrane intact.


100μ
1
RC
Sc
2
2μ
2μ
5
5μ
6

## 3.2 METHODS OF HANDLING AND COLLECTING EGGS

Collecting large numbers of eggs of known age, was essential for much of the experimental work. The following procedures were used to facilitate collection and timing of these eggs. All eggs used in the following experiments were obtained from moths reared from field-collected pupae.

### *3.2.1 EGGS FOR STOCK USE*

Rape plants were used as the ovipositional substrate in all experiments in which large number of eggs of unknown age were required. Moths were placed in cages containing four rape plants and subjected to a photoperiod of 16L:8D. Eggs were collected once a day at the end of the dark period, by removing leaves containing eggs clusters. Often it proved necessary to break the clusters into smaller units. The adhesive which binds the eggs to the leaves was softened with distilled water and individual eggs were removed by gentle pushing with a camel hair brush.

### *3.2.2 COLLECTING ACCURATELY-TIMED EGGS*

A stock culture of male and female moths was caged in a growth chamber at 20° C and approximately 60% RH (for some experiments a culture was maintained at 15° C). The moths were fed a 10% honey and water solution from wicked containers. The solution was changed twice a week. Since *M. configurata* generally oviposits at night, I reversed

the normal photoperiod so that darkness occurred between 8:00 a.m. and 4:00 p.m. This eased collection of eggs.

Females prefer a rough substance for oviposition, but, given no choice, will oviposit on almost any surface. Plastic sandwich wrap was avoided by females probably because they were unable to find a purchase on this material. If the cage is lined with this material the moths show strong ovipositional preference for paper toweling or other rough textured materials. Paper toweling was chosen as the ovipositional substrate because of its availability and ease of handling. The toweling was folded in such a manner that it was able to stand on its own. It was introduced into the cage approximately one hour after dark and replaced 1 3/4 h later.

Once the paper toweling was removed, the egg clusters were examined at X12 under a binocular microscope and were separated into groups of the desired number with a razor blade. The strips containing the eggs were quickly examined and any damaged eggs were discarded. Each strip was then placed in a clean, 1mm cap vial, ready to be used in the experiment. The top of each vial was covered with plastic screening, held secure by a rubber band. Any eggs remaining from an egg cluster were placed in a vial marked to indicate their origin and were used to check fertility for that group.

## 3.3 Viability and size of field-collected versus laboratory-deposited eggs.

The main purpose of this experiment was to determine if laboratory rearing and handling techniques influenced either egg viability or numbers laid when compared to eggs deposited under field conditions.

### 3.3.1 *MATERIALS AND METHODS*

A laboratory culture of adult moths was maintained in a growth chamber at 20° ± 0.5° C and approximately 60% RH. The photoperiod maintained was 16L:8D, a close approximation of field conditions at the time of the experiment. The moths were provided with potted rape plants for oviposition, these being at approximately the same stage of development as were field sown plants. Ten egg clusters of unknown age were removed at random from the caged culture by removing the leaf supporting the cluster. Each egg cluster was then divided carefully into groups of 15 by a technique previously mentioned (3.2) and placed in 1mm cap vials. When less than 15 eggs remained, the remaining group was also placed in a 1mm cap vial. Eggs from each cluster were separate so that mortality and number of eggs could be recorded for each cluster.

Field samples of eggs were studied in a field of rape near Lacombe, Alberta. Ten egg clusters, of unknown age were located and counted and the leaf supporting each was encased in a fiberglass screen having a mesh size of 16 threads per cm to prevent access of predators and parasites and larval escape. These cages were inspected

twice daily and the number of hatched larvae recorded for each cage.

### 3.3.2 *RESULTS*

The results of this experiment are recorded in Table 1. Egg viability did not differ significantly between the two groups but the average size of the laboratory clusters was larger than that of the field clusters.

## 4. TEMPERATURE THRESHOLDS FOR EMBRYOGENESIS

Insect embryogenesis is influenced by many external factors which, acting separately or together restrict developmental potential. Within the range of each of these factors are arbitrary points designated as thresholds. Continual exposure of an embryo to these factors beyond these points eventually results in its death.

One of the most important and easily studied factors influencing embrybgenesis is temperature. Insects, being poikilothermic, are greatly affected by the temperature surrounding them. However, only the egg and the quiescent pupal stage are unable to move to a different microclimate to avoid temperature extremes. For this reason eggs and non-mobile pupae are superior to other stages for studies of temperature thresholds.

### 4.1 The developmental threshold

The developmental threshold is the temperature at which, on the

TABLE 1 A COMPARISON OF SIZE OF EGG CLUSTERS AND VIABILITY BETWEEN LABORATORY-AND FIELD-COLLECTED EGGS

| Field Collected | | | Laboratory Collected | | |
|---|---|---|---|---|---|
| # of eggs | # hatched | per centage hatch | # of eggs | # hatched | per centage hatch |
| 56 | 53 | 94.64 | 130 | 128 | 98.46 |
| 78 | 75 | 96.15 | 189 | 186 | 98.41 |
| 86 | 85 | 98.84 | 254 | 247 | 97.24 |
| 42 | 38 | 90.48 | 127 | 123 | 96.85 |
| 28 | 28 | 100.00 | 95 | 94 | 98.95 |
| 187 | 186 | 99.46 | 102 | 99 | 97.06 |
| 119 | 118 | 99.16 | 79 | 73 | 92.96 |
| 97 | 95 | 97.94 | 163 | 159 | 97.55 |
| 63 | 61 | 96.82 | 129 | 129 | 100.00 |
| 52 | 50 | 94.34 | 197 | 190 | 96.45 |
| 80.8* | 78.9 | 96.82 | 146.5* | 142.8 | 97.03 |

*means significantly different at the 1% level based on T-tests.

descending scale, development definitely ceases, and at which, on the ascending scale, development is initiated (Peairs, 1927). Knowledge of this threshold is vital if one is attempting to predict development on the basis of temperature. An experiment was conducted to determine this threshold for embryos of *M. configurata*. Correlative histological studies were also made.

### 4.1.1 MATERIALS AND METHODS

Eggs used in this experiment were collected from batches of over 100 eggs deposited by adult moths maintained at 15 ± 0.5° C. Eggs were placed at the experimental temperatures when less than 30 minutes old, thus insuring (based on Rempel's 1951 paper) that no prior development had occurred.

After collection, the eggs were immediately transferred to temperatures of 0 ± 0.5° C, 2 ± 0.5° C and 4 ± 0.5 ° C maintained in separate growth chambers. Two separate clusters of eggs were placed at each temperature. The eggs from each cluster were divided into 20 groups of five eggs each, using a technique previously mentioned, each group of five being placed in a separate 1 mm cap vial (The eggs of the 2 clusters were kept separate). All but two vials were placed on a platform in a 160 mm desiccator containing a saturated salt solution ($KNO_3$) (Winston and Bates, 1960) used to maintain the humidity at about 96%. The eggs of one of the remaining vials were fixed immediately in hot, alcoholic Bouins solution while the other vial was removed and placed at room temperature to determine if all its contained eggs were fertile.

Hot alcoholic Bouins was poured into one vial from each group daily

for the first five days. After that time eggs were fixed at five day intervals. Standard histological techniques were used to prepare the eggs for staining with Delafield's Haemotoxylin and Mallory's Triple Stains.

The experiment was concluded when anatomical signs of development were found in 2 or more eggs from both vials.

### *4.1.2 RESULTS*

The results of this experiment are contained in Table 2.

The temperature developmental threshold for embryogenesis of M. *configurata* is between 0 and 2° C.

Eggs at 4° C showed recognizable development after 10 days and at 2° C after 15 days. No sign of development occurred at 0° C even after 40 days.

TABLE 2 NUMBERS OF EGGS PER VIAL SHOWING SIGNS OF DEVELOPMENT AT VARIOUS TEMPERATURES

TEMPERATURES °C (max. hatch is 5)

| | 0 | | | | | | | | 2 | | | | | | | | 4 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | 1 | 2 | . . . | 5 | 10 | 15 | . . | 30 | 40 | | 1 | 2 | . . . | 5 | 10 | 15 | | | 1 | 2 | . . . | 5 | 10 | |
| Group 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | 0 | 0 | 0 | 0 | 3 | | | | 0 | 0 | 0 | 2 | | |
| Group 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | 0 | 0 | 0 | 0 | 4 | | | | 0 | 0 | 0 | 4 | | |

## 4.2 The hatching threshold

The hatching threshold is the lowest temperature at which hatching of a fully developed larva can occur (Johnson, 1940). Knowledge of this temperature can be used in planning larval surveys. For example, the pale western cutworm, *Agrotis orthogonia* Morr. (Lepidoptera:Noctuidae) over-winters as a fully developed larva within the egg (Jacobson and Blakelay, 1958). If the hatching threshold was known for this insect, it would be possible to implement control measures before damage occurred.

The egg of *M. configurata* is an excellent subject for this type of experiment because darkening of the head capsule always indicates that development is complete and that hatching is soon to follow.

### *4.2.1 MATERIALS AND METHODS*

Three egg clusters, less than one day old, and each containing in excess of 150 individuals, were collected over a period of two days from a stock culture (see 3.1). Each cluster was divided into groups of 50. Surplus eggs from each cluster were placed in 1mm cap vials and labelled according to their cluster of origin. These eggs were used as spares to be substituted for any infertile eggs in the experimental group. Each of these nine groups of 50 eggs was placed in a 4mm glass cap vial and labelled so that groups from individual clusters could be identified. All vials were then placed on a plat-

form, at 20° C in a 160mm desiccator which had been partially filled with distilled water to raise the humidity to greater than 90%.

The eggs were observed closely and were allowed to develop to the black head capsule stage. Then, to prevent larval escape, the tops of the vials were covered with a piece of plastic screening, secured by a rubber band. One vial from each egg cluster was then quickly transferred to identical, water-filled, desiccators located in incubators set at temperatures of 7.5 ± 0.5° C, 5 ± 0.05° C and 2.5 ± 0.5° C. The temperatures of the incubators were continually monitored using hygrothermographs. The eggs were observed twice daily and the total number of hatched eggs recorded. The eggs were retained at the experimental temperature for a maximum of 30 days after which they were returned to 20° C to determine if the remaining eggs would hatch.

*4.2.2 · RESULTS*

The results of this experiment are summarized in Table 3. The first group of eggs to hatch were those at 7.5° C. The lowest experimental temperature at which eggs hatched was 5° C. No eggs hatched at 2.5° C even after 30 days and these failed to hatch even after being returned to 20° C. Examination of these eggs showed the embryos to have died and dried up. There was a greater percentage hatch at 7.5° C than at 5° C (average 7.5° C - 92.2%, and 5° C - 66%).

TABLE 3

The Hatching Threshold for Eggs of M. *configurata*

| | Temperature ° C | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | | | 5 | | | 7.5 | | |
| Replicate number | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Number hatching per replicate of 50 | 0 | 0 | 0 | 31 | 33 | 35 | 47 | 44 | 48 |

## 4.3 The Developmental-hatching threshold

The developmental-hatching threshold is the lowest temperature at which complete development from fertilization to eclosion can occur (Johnson, 1940). This threshold is the most important of those so far discussed, because, for some species, it can be used as an aid in determining distribution. Obviously, areas in which the daily high temperature is always below this threshold, for a particular insect, will not have that insect present.

In the Peace River and Manning areas of Alberta it is not uncommon for the daily high temperature early in the period of adult emergence (late June and early July), to be 10° C or lower. This experiment was designed to determine the lowest temperature at which complete development of *M. configurata* eggs to eclosion could occur. Three different relative humidities were used to show if humidity had any influence on development. Previous research had shown that complete embryogenesis could occur at 10° C but not at 5° C.

### 4.3.1 *MATERIALS AND METHODS*

Eggs were collected over a period of three days from a culture of adults maintained at 15 ± 0.5° C and 60% RH. The methods used for collecting and handling eggs were identical to those described in 3.2.2 with the exception that they were collected when less than one hour old to insure that little, if any, development had occurred.

After collection, the eggs were divided into 10 groups of 30, each group being placed in a 1mm cap vial. One of the groups was placed at 20 ± 0.5° C and left for the remainder of the experiment as a test of egg viability. The remaining nine groups formed the first of three replicates. Each of the nine vials was placed under a different experimental condition. Vials were placed at temperatures of 6.5 ± 0.25° C, 7.5 ± 0.25° C, and 8.5 ± 0.5° C and at relative humidities of about 0%, 60% and 98% for each temperature. Temperatures were maintained in an incubator (6.5° C) in a refrigerated water bath (7.5° C) and in a growth chamber (8.5° C). Relative humidity was controlled in 6mm cap vials using saturated salt solutions ($P_2O_5$ for 0%, $Na_2Cr_2O_7\ H_2O$ for 60% and $K_2SO_4$ for 98%) (Winston and Bates, 1960). Each of the two remaining daily collections were treated in an identical manner.

The eggs were observed daily for signs of development. When embryos reached the black head capsule stage, observations were made every two hours until hatching was complete.

### 4.3.2 *RESULTS*

The results of this experiment are summarized in Table 4. The numbers recorded are totals for the three replicate listed in Appendix II. Percentage hatch for each control was 90% or greater. In the experimental groups, hatching occurred only at 8.5° C and 98% RH (see Table 4). Development to the black head capsule stage occurred in some individuals at 8.5° C and 60% RH and at 7.5° C and 98% RH (Appendix II). Dissection

TABLE 4

The Developmental-Hatching Threshold of eggs of M. *configurata* and the Effects of Relative Humidity on it

| Temperature ° C | Number Hatching (maximum 90) | | |
|---|---|---|---|
| | 0% RH | 60% RH | 98%RH |
| 6.5 | 0 | 0 | 0 |
| 7.5 | 0 | 0 | 0 |
| 8.5 | 0 | 0 | 20 |

of a portion of these embryos several days later revealed no outward signs of disorder.

## 4.4 The high temperature developmental-hatching threshold

*M. configurata* has a reported geographic range that spans vastly different climatic regions from Keg River in northern Alberta to Mexico City, Mexico. It is thus highly likely that this species occurs in several biotypes having different climatic preferences. The biotype occurring in central and northern Alberta is adapted to cool springs, warm short summers, and long cold winters. In Alberta the eggs are exposed to temperatures ranging from 5° C to the mid 20's. However, in the southern part of its range, it is likely that the eggs of *M. configurata* are exposed to a much higher range of temperatures.

Fry and Surber (1971) reported that temperatures in Arizona cotton-fields (well within *M. configurata's* range) reached 35° C or more for periods of over 16 hours per day and 40° C for over eight hours a day (one of the species they studied, the salt march caterpiller (*E. acrea*) is an occasional pest of rape in Alberta and is often found in conjunction with *M. configurata*).

The purpose of this experiment was to determine the high temperature developmental-hatching threshold for *M. configurata* and to find out whether this cold adapted biotype retains some resistance to high temperatures (30° C and above).

### 4.4.1 MATERIALS AND METHODS

This experiment was performed essentially like the previous one (4.3) except that temperatures of 30 ± 0.5° C, 31.5 ± 0.5° C, 32.5 ± 0.5° C and 33.5 ± 0.5° C were used. Temperatures of 30° C and 33.5° C were maintained in a heated water bath and of 31.5° C and 32.5° C in incubators. Humidities were maintained using saturated salt solutions of $P_2O_5$ for 0% RH, $NH_4NO_3$ for approximately 60% and $K_2Cr_2O_7$ for 98% RH (Winston and Bates, 1960).

### 4.4.2 RESULTS

The results of this experiment are summarized in Table 5. The listings are the totals of the three replicates of 30 listed in Appendix III. Unlike the previous experiment, relative humidity did not have a significant effect on hatching. Of the four temperatures tested, hatching occurred only at 30° C regardless of humidity. Some development occurred in eggs at all other experimental temperatures. All eggs (with the exception of some probably infertile ones) developed the characteristic brown pigment of the equator and micropylar areas.

Eggs at 32.5° C developed to the point where the larvae were clearly visible through the chorion, however, sclerotization and pigmentation did not occur.

TABLE 5

The High Temperature Developmental Threshold of Eggs of M. *configurata* and the Effects of Relative Humidity on it

| Temperature in ° C | No. Hatching (maximum 90) | | |
|---|---|---|---|
| | 0% RH | 60% RH | 98% RH |
| 30 | 84 | 82 | 84 |
| 31.5 | 0 | 0 | 0 |
| 32.5 | 0 | 0 | 0 |
| 33.5 | 0 | 0 | 0 |

# 5.0 THE EFFECTS OF CONSTANT TEMPERATURE AND RELATIVE HUMIDITY ON EMBRYOGENESIS

## 5.1 The Relation between Constant Temperature and 0%, 60% and 98% Relative Humidity and their Effects on Developmental Rate.

When dealing with an insect pest, knowledge of the effects of climatic factors particularly those of temperature, on various stages of its lifecycle is imperative if adequate predictions of outbreaks are to be made and if successful control procedures are to be implemented. For example, timing of the various surveys used in estimating the abundance of M. *configurata* (see Introduction) must take into consideration the effects of climate. A fall pupal survey begun when there are still large numbers of active larvae would be inaccurate. The most accurate way to determine the individual effects of various field conditions on a particular stage of a pest is to duplicate every possible combination of those conditions in the laboratory. This, however, is usually impossible to do or is too time consuming. The usual alternative (although it lacks the same degree of accuracy) is to use a range of constant temperatures, encompassing the range found in nature and to extrapolate from these results, to development in the field.

Few authors have attempted to evaluate the effects of relative humidity acting in conjunction with constant temperature on insect embryogenesis. This is rather surprising when one considers the effects relative humidity can have on development (Buxton, 1931; Ludwick, 1945; and Bursell, 1974). Since M. *configurata* has a large

geographic range, it is probably exposed to a considerable range of relative humidities. This experiment was conducted to determine the effects that relative humidity might have on embryogenesis under constant temperature conditions.

### 5.1.1 *MATERIALS AND METHODS*

Ten different temperatures combined with three different relative humdities were used to determine the effect of temperature and relative humidity on embryogenesis. The lowest temperature used was 8.5° C (the developmental hatching threshold; see section 4.3); the highest 30° C (the high temperature developmental threshold; see section 4.4). The remaining temperatures began at 10° C and increased at 2.5° C intervals. Humidities of approximately 0, 60 and 98% were maintained in 6mm cap vials using various saturated salt solutions (Winston and Bates, 1960). Growth chambers were used to control temperatures of 8.5° C, 12.5° C, 20 ° C, and 25° C, and incubators for the remainder. The temperature in each incubator was monitored periodically throughout the experiment and in no case was it found to vary more than $\pm$ 0.5° C when the door was closed. For the majority of experiments, the doors were only opened briefly, once a day, to check on egg development. When the door was opened the temperature returned to equilibrium in less than 30 minutes after the door was closed.

Dry $P_2O_5$ was used to provide 0% RH, and $KNO_3$ to provide 98% RH at all temperatures used. NaBr . $2H_2O$ was used to approximate 60% RH

for temperatures of 10° C, 12.5° C, 15° C, 17.5° C and 20° C, $Na_2Cr_2O_7 \cdot H_2O$ for a temperature of 8.5° C and $NH_4NO_3$ for the remainder (Winston and Bates, 1960). The saturated salt solutions were made up and placed at the desired temperature 30 days prior to being used to allow them to stabilize. Humidities in the chambers were checked prior to, during and after the experiment and were always found to be within 6% of the desired humidity.

The eggs used in this experiment were collected by the methods previously described (Section 3.3.2) from a culture of adults kept at 20 ± 0.5° C and 60% RH. Thirty eggs were placed in each vial. These vials were placed at the experimental conditions within one hour of collection. The experiment was replicated three times. Early in the experiment, it became apparent that insufficient numbers of eggs could be collected at any one time to complete a replicate. Instead, the eggs of each collection were used in replicating the three relative humidity treatments used at each temperature.

The eggs were examined once a day until they had reached the black head capsule stage at which time recorded observation were made at two hour intervals until hatching was complete.

### 5.1.2 RESULTS

The results of these experiments are given in Appendix IV and are summarized in Table 6 and in Figs. 7-9. Hatching occurred at all temper-

TABLE 6 RELATIVE HUMIDITY - The Effect of Temperature and Three Relative Humidities on the Mean Development Time and on the Development Rate of Eggs of M. *configurata*.

0% RH**

| Temperature ° C | Mean Development Time in Hours | Mean % Development Per Hour |
|---|---|---|
| 8.5 | X | X |
| 10 | X | X |
| 12.5 | X | X |
| 15 | 259.70 | 0.00385 |
| 17.5 | 226.49 | 0.00441 |
| 20 | 143.28 | 0.00698 |
| 22.5 | 124.98 | 0.00800 |
| 25 | 108.13 | 0.00923 |
| 27.5 | 91.7 | 0.0109 |
| 30 | 87.97 | 0.0114 |

X - Did not complete development to eclosion.

** based on 3 replicates of 30 eggs per replicate

Table 6 (Cont.)

60% RH**

| Temperature ° C | Mean Development Time in Hours | Mean % Development Per Hour |
|---|---|---|
| 8.5 | X | X |
| 10 | 576.19 | 0.00173 |
| 12.5 | 462.65 | 0.00216 |
| 15 | 252.32 | 0.00396 |
| 17.5 | 220.71 | 0.00453 |
| 20 | 135.14 | 0.00740 |
| 22.5 | 118.80 | 0.00842 |
| 25 | 100.92 | 0.00991 |
| 27.5 | 83.05 | 0.0120 |
| 30 | 78.71 | 0.0127 |

98% RH**

| Temperature ° C | Mean Development Time in Hours | Mean % Development Per Hour |
|---|---|---|
| 8.5 | 879.7 | 0.00114 |
| 10 | 564.59 | 0.00177 |
| 12.5 | 458.39 | 0.00218 |
| 15 | 250.04 | 0.00399 |
| 17.5 | 218.88 | 0.00457 |
| 20 | 133.02 | 0.00752 |
| 22.5 | 116.75 | 0.00856 |
| 25 | 98.60 | 0.0101 |
| 27.5 | 81.13 | 0.0123 |
| 30 | 76.86 | 0.0130 |

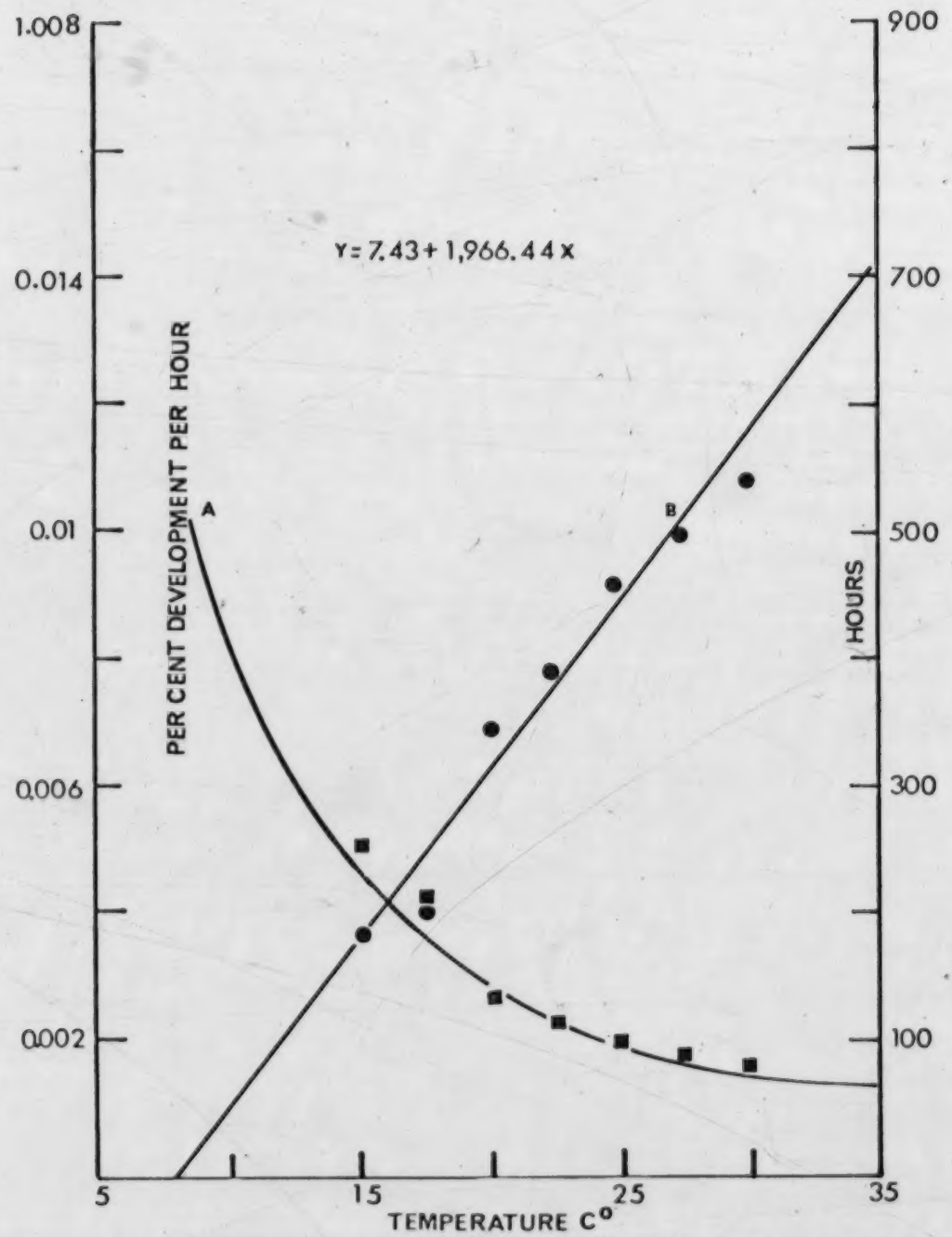



Fig. 7. The developmental curve: A, and the velocity line: B, for 0 percent relative humidity, for embryogenesis of *M. configurata*

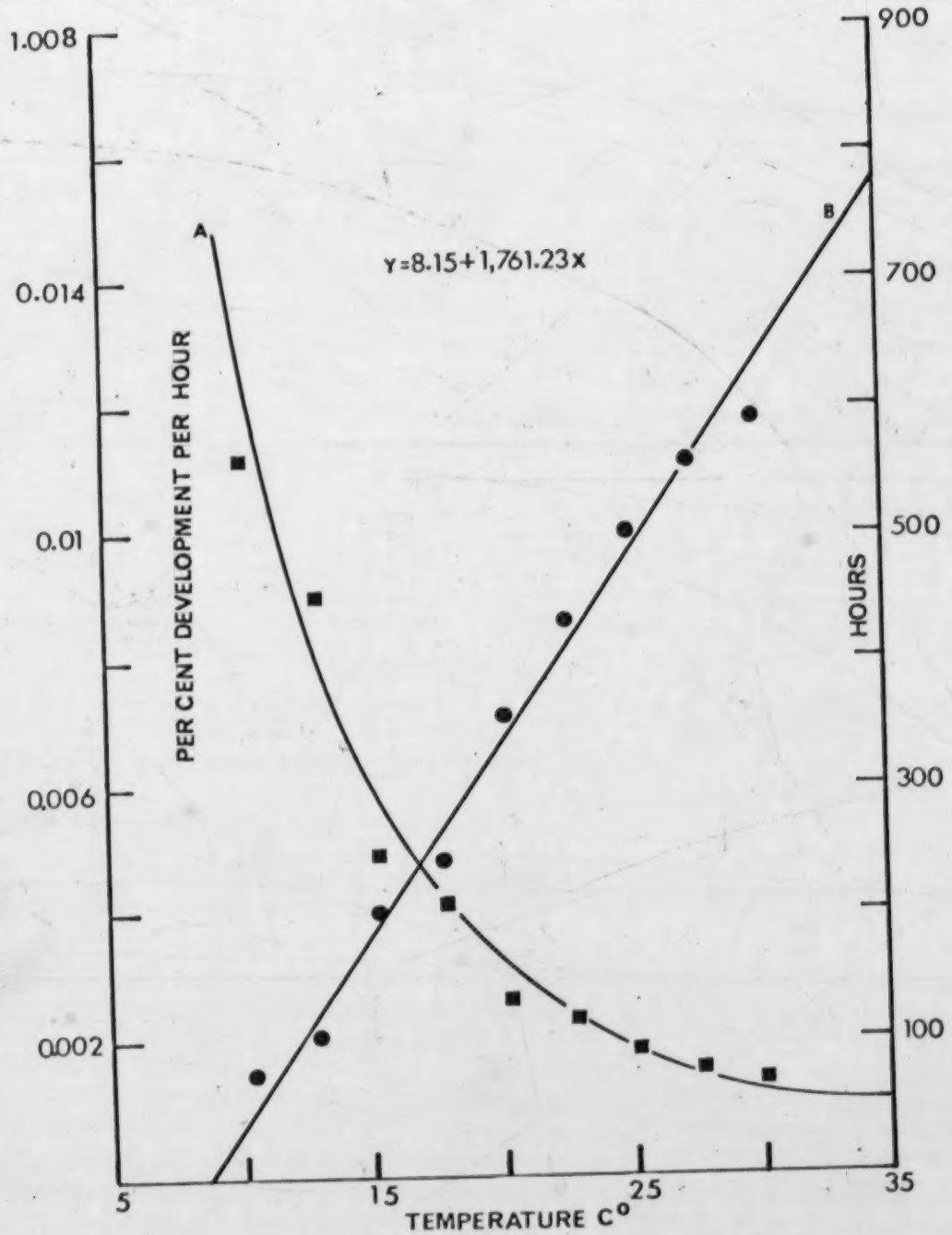



Fig. 8. The developmental curve: A, and the velocity line: B, for 60 percent relative humidity, for embryogenesis of *M. configurata*

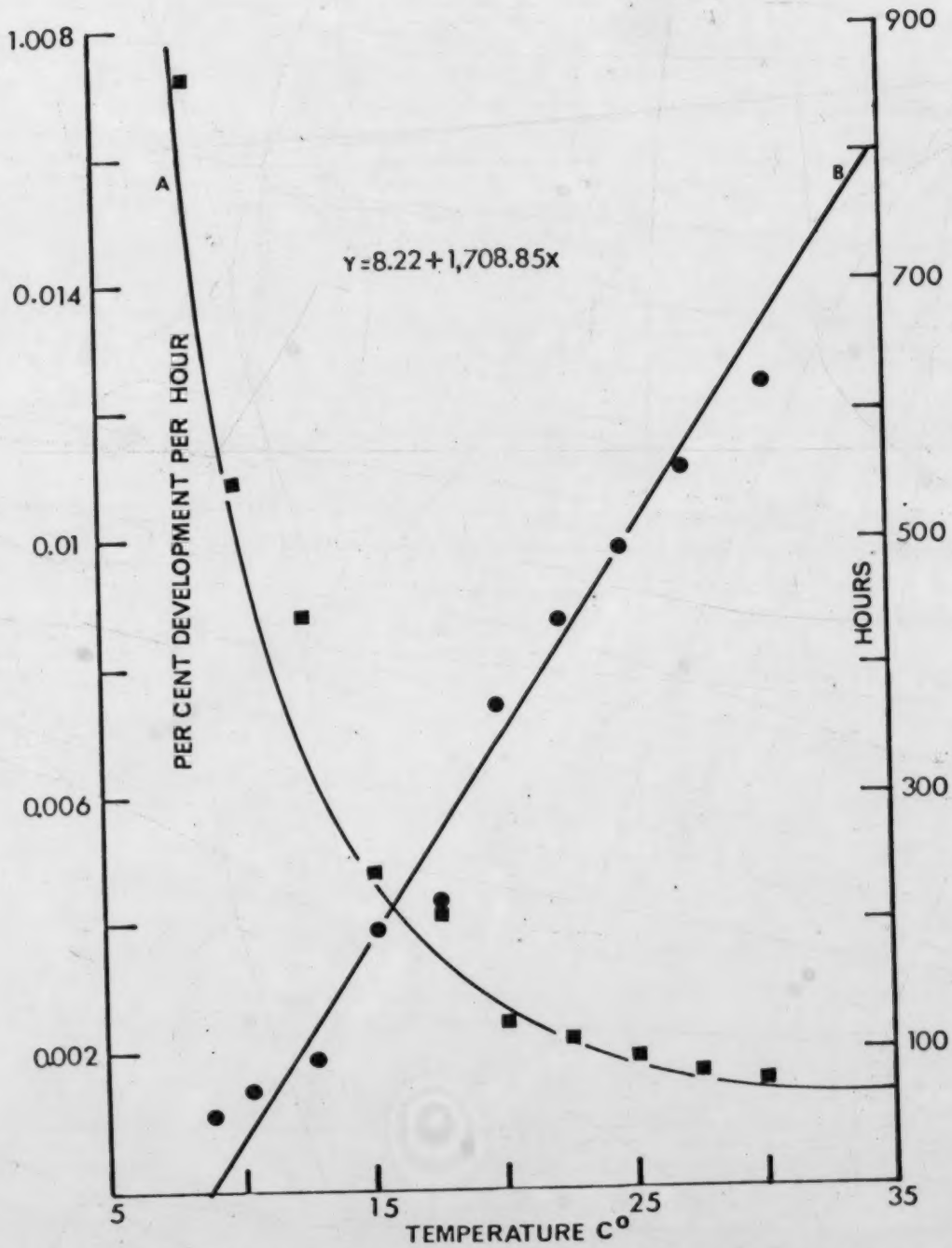



Fig. 9. The developmental curve: A, and the velocity line: B, for 98 percent relative humidity, for embryogenesis of *M. configurata*.

atures tested. The rate of development (the reciprocal of the total development time measured in hours) showed a strong positive correlation with temperature (Correlation coefficients of 0.978, 0.989 and 0.982 were recorded for 0%, 60% and 98% RH respectively). The fastest development occurred at 30° C; the slowest at 8.5° C.

Humidity had a considerable effect on development. Development but no hatching occurred at 0% RH for temperatures of 12.5° C and lower, and at 60% RH for 8.5° C. Hatching always occurred first in those eggs at 98% RH followed by those at 60% RH and 0% RH.

Figures 7 to 9 demonstrate the effects of relative humidity and temperature on development. The straight lines (B) are drawn on the basis of the linear regression equations $Y=7.43 + 1,966.44x$; $Y=8.15 + 1,761.23x$ and $Y=8.22 + 1,708.85x$ for 0%, 60% and 98% RH respectively. These lines are the velocity lines and represent the percentage of total development that occurs during one hour at that temperature. The points on and around these lines are the calculated values based on the reciprocal of the mean development time (listed in Table 6).

The hyperbolic curve (A) is based on the mean development time in hours at the experimental temperatures and humidities indicated. The squares on the graph represent the mean development time in hours for that temperature and humidity. The means used in Table 6 and in Figs 7-9 are taken from the three replicates listed in Appendix IV.

## 5.2 The effects of constant exposure to a temperature of 35° C on development of eggs of different ages

The purpose of this experiment was to determine the tolerance of eggs of *M. configurata* to extended exposure to high temperature. Results of preliminary experiments had suggested that *M. configurata* is imperfectly adapted to the climatic regime found in the prairie provinces (Putnam, 1972). To perfectly adapt to this climate the pupal population of *M. configurata* should have an obligatory diapause. This however is not always the case since flights of newly-emerged adults have been trapped in blacklight traps in late fall (Philip, 1972), and some field-collected pupae have been shown to develop without exposure to cold (Philip, 1973). These observations suggest that at least part of the population has either a facultative diapause or lacks it completely. Preliminary experiments have shown that larvae of *M. configurata* can respond to photoperiod and temperature (Putnam, 1972). The existence of this partial second generation in the prairie provinces suggests that *M. configurata* may be bi-or multivoltine in more southerly parts of its range.

The main purposes of this experiment were to determine if the eggs of *M. configurata* demonstrate a tolerance to high temperature and to discover the length of exposure required to produce 50 and 95% mortality. A temperature of 35° C was chosen for two reasons; first, because previous research (see Section 5.3) had shown that this was above

the upper developmental-hatching threshold and second, because temperatures of 35° C or greater for periods over 16h have been recorded in Arizona cottonfields (Fry and Suber, 1971), well within M. *configurata*'s range. Their study on the effects of high temperature on embryo mortality included *E. acrea* which is often found in conjunction with M. *configurata* and which has a similar life cycle in Alberta (Beirne, 1971).

### 5.2.1 MATERIALS AND METHODS

Eggs used in this experiment were collected from a culture of adults reared at 20 ± 0.5° C, by previously described methods (see section 3.2).

Eggs of four different ages (3h, 24h, 48h and 96h) were exposed to 35° C continuously for periods of 13, 20, 30, 45 and 67.5h. Six replicates of 20 eggs were used for each of the four different ages.

The eggs were collected daily when less than 2h old, divided into groups of 20 and each group was placed in an uncapped, 1mm vial. The vials were divided into groups of six forming the replicates, and the remaining vials were used as a check to determine natural mortality. The eggs that were to be used later were left at 20 ± 0.5° C and approximately 60% RH until they reached the desired age. When this occurred the eggs were placed in a 160mm desiccator located in an incubator set at 35° C. The humidity in the desiccator was maintained at approximately 60% with a saturated salt solution of $NH_4NO_3$

(Winston and Bates, 1960).

The temperature in the incubator was monitored periodically throughout the experiment and never varied more than 0.5° C. The temperature in the desiccator and within the vials was checked periodically and showed little variance (0.25° C) from that of the incubator. The incubator was opened only when it was necessary to place or remove vials.

Once the treatment was completed, the eggs in the vials were returned to 20 ± 0.5° C and 60% RH. The tops of the vials were covered with plastic screening to prevent larval escape. The numbers hatching were recorded twice daily. Abbot's formula (Abbot, 1925) was used to determine the net percentage mortality. Natural mortality was considered to be the highest mortality that was not a function of the treatment.

The resulting sigmoidal, dose mortality curves were transformed into straight lines using probit analysis (Bliss, 1935). This transformation allows direct comparison to be made between dosage and the percent mortality that can be expected. Variance analysis was used to determine the amount of variance caused by replication and error.

### 5.2.2 *RESULTS*

The results of this experiment are given in Appendix V and are summarized in Figures 10 to 13 and in Tables 7 to 10.

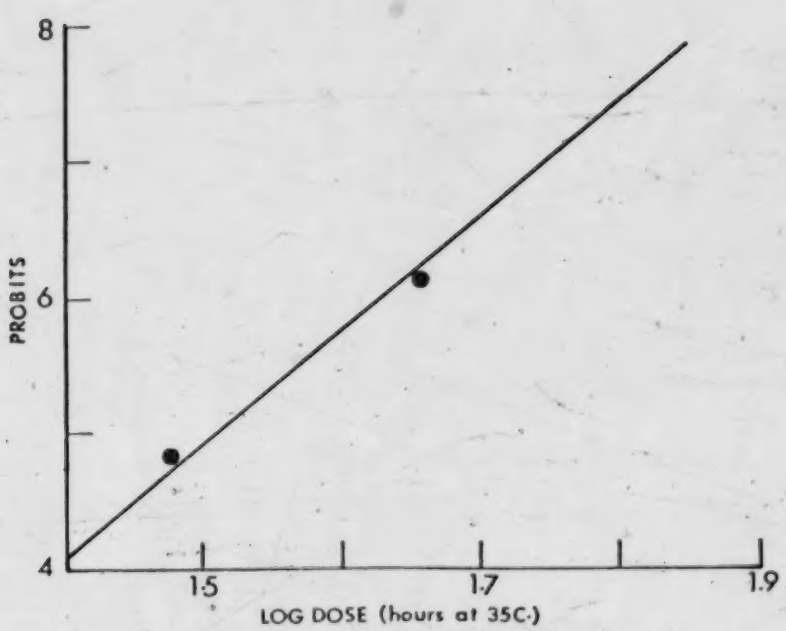



Fig. 10. Probit regression line showing the mortality, of various dosages of 35°C. on eggs 3 hours old.

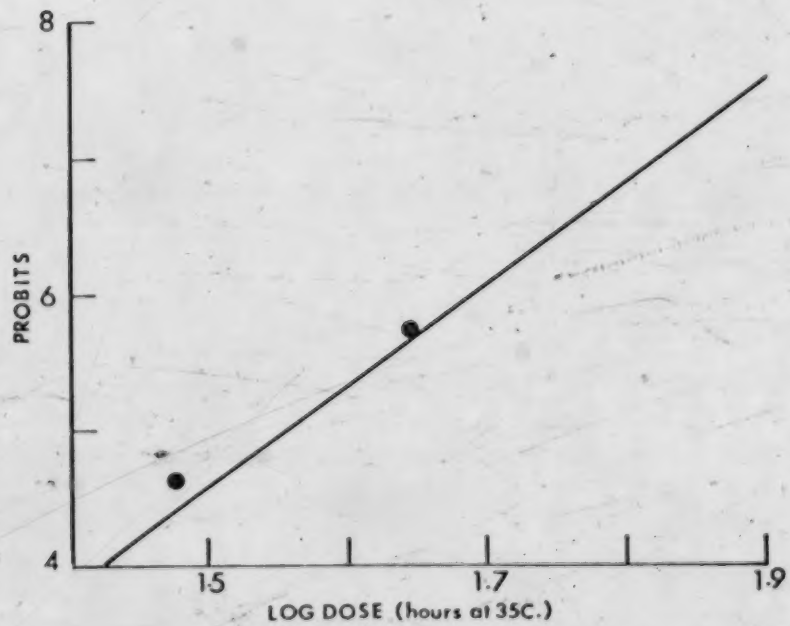



Fig. 11. Probit regression line showing the mortality, of various dosages of 35° C on eggs 24 hours old.

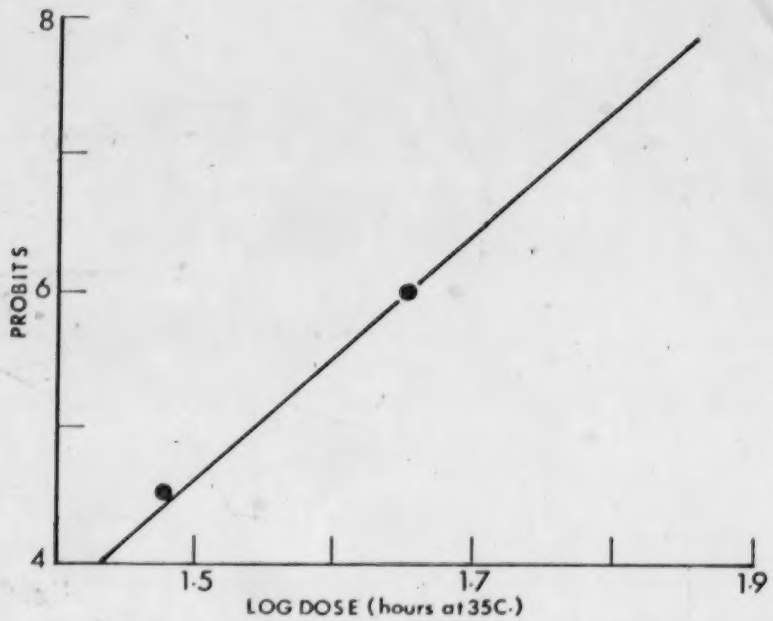



Fig. 12. Probit regression line showing the mortality, of various dosages of 35° C. on eggs 48 hours old.

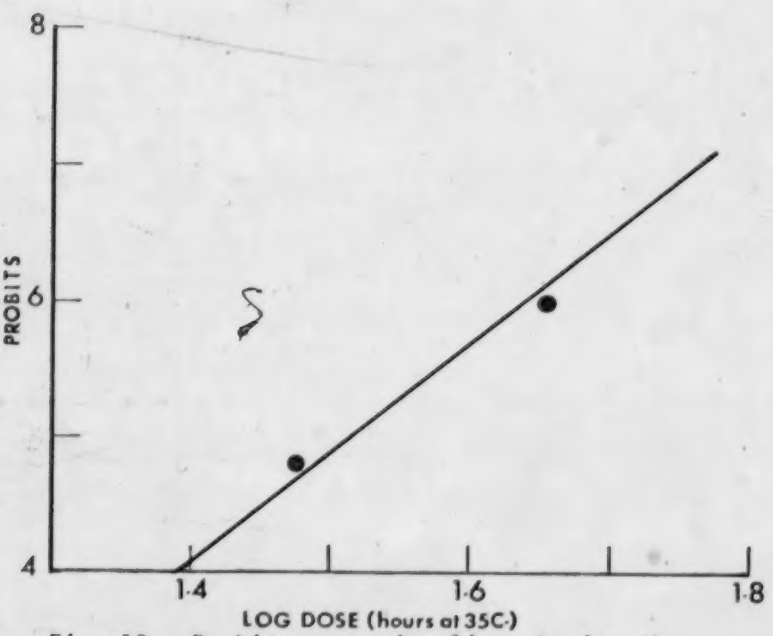



Fig. 13. Probit regression line showing the mortality, of various dosages of 35° C. on eggs 96 hours old.

TABLE 7 The Effect of Continuous Exposure to 35° C on Eggs of M. *configurata* Three Hours old.

| No. of Individuals Tested | Length of Treatment in Hours | Net% Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 13 | 0 | |
| 120 | 20 | 0 | |
| 120 | 30 | 32.35 | 4.5407 |
| 120 | 45 | 84.31 | 6.0069 |
| 120 | 67.5 | 100 | |

Treatment F-value 433.06 (F at 1% 4.43) with 4 DF

Between Treatment F-value 1.72 (F at 5% 2.71) with 20 DF

Mean number of hours of exposure at 35° C required to produce 50% and 95% mortality are: 34.45 ± 1.40 and 51.27 ± 3.55 respectively. Variance analysis showed that variation in pereentage mortality was a function of treatment.

TABLE 8 The Effect of Continuous Exposure to 35° C on Eggs of M. *configurata* 24 Hours old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 13 | 0 | |
| 120 | 20 | 0 | |
| 120 | 30 | 41.66 | 4.7895 |
| 120 | 45 | 84.26 | 6.0051 |
| 120 | 67.5 | 100 | |

Treatment F-value 404.1 (F at 1% level 4.43) with 4 DF

Between Treatment F-value 0.77 (F at 5% level 2.71) with 20 DF

Mean number of hours of exposure to 45° C required to produce 50% and 95% mortality are 33.19 ± 3.49 and 48.31 ± 3.34 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

TABLE 9 The Effect of Continuous Exposure to 35° C on Eggs of M. *configurata* 48 Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 13 | 0 | |
| 120 | 20 | 0 | |
| 120 | 30 | 44.06 | 4.8516 |
| 120 | 45 | 87.28 | 6.1407 |
| 120 | 67.5 | 100 | |

Treatment F-value 805 (F at 1% level 4.43) with 4 DF

Between treatment F-value 0.82 (F at 5% level 2.71) with 20 DF

Mean number of hours of exposure to 35° C required to produce 50% and 95% mortality are 32.8 ± 1.33 and 50.03 ± 3.4 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

TABLE 10 The Effect of Continuous Exposure to 35° C on Eggs of M. *configurata* 96 Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 13 | 0 | |
| 120 | 20 | 0 | |
| 120 | 30 | 34.775 | 4.6093 |
| 120 | 45 | 76.97 | 5.7388 |
| 120 | 67.5 | 100 | |

Treatment F-value 426.78 (F at 1% level 4.43) with 4 DF

Between Treatment F-value 0.05 (F at 5% level 2.71) with 20 DF

Mean number of hours of exposure to 35° C required to produce 50% and 95% mortality are 35.17 ± 1.11 and 54.95 ± 4.14 hours respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

The mean time required in hours to produce 50% mortality are 34.45 ± 1.4, 33.19 ± 3.49, 32.8 ± 1.33 and 35.17 ± 1.11 h for 3h, 24h, 48h and 96h old eggs respectively; and to produce 95% mortality are 51.27 ± 3.55h, 48.31 ± 3.34h, 50.03 ± 3.4h and 54.95 ± 4.14h for 3h, 24h, 48h and 96h old eggs respectively. From this data it can be concluded that exposures of greater than 37h to a temperature of 35° C will result in 50% or greater mortality and exposures of 60h will result in 95% or greater mortality, to eggs of M. *configurata* regardless of their age.

Figures 10 to 13 show the relationship existing between dosage (hours at 35° C) and mortality. The line representing this relationship was calculated using the provisional probits which included probits for both 0% and 100% mortality (since both conditions occurred in the experiment). The data used to draw these lines are shown in Tables 7 to 10.

Variance analysis and the resulting F-values showed that length of exposure was highly significant in determining mortality. F-values of 433.06, 404.1, 805 and 426.78 (F at 1% level 4.43) were calculated for 3h, 24h, 48h and 96h exposures respectively. Between replicates, the F-values were not significant with 1.72, 0.77, 0.82 and 0.05 (F at 5% level 2.71) for 1h, 24h, 48h had 96h exposures respectively.

## 5.3 The effects of constant exposure to a temperature of 5° C on eggs of different ages

When I began this research I found that M. *configurata* was difficult to rear continuously in the laboratory. Part of the problem was feeding the large number of larvae produced. The main purpose of this experiment was to determine the length of time eggs of M. *configurata* could be stored at 5° C without causing excessive mortality. The temperature of 5° C was chosen for two reasons: (1) under natural conditions, the eggs are often exposed to this temperature or lower for considerable periods of time and (2) 5° C is below developmental-hatching threshold, but above the developmental threshold. A by-product of this research was the determination of the length of exposure to 5° C required to produce 50% and 95% mortality.

### 5.3.1 *MATERIALS AND METHODS*

The procedures used in this experiment were identical to those of section 5.2.1 with the following exceptions:
(1) The exposure was to 5° C; (2) $NaBr \cdot 2H_2O$ (Winston and Bates, 1960) was used to maintain approximately 60% RH and (3) exposure times of 30, 45, 67, 101, 151, 227, 340, 510 and 765h were used. Additional times of 273h was used for eggs 48h old and 83h old for 3h old when initial mortality was high.

5.3.2 *RESULTS*

As in the previous experiment, probit analysis was used to transform sigmoidal dosage mortality curves into straight lines (Bliss, 1935). Variance analysis was used to determine the sources of variation and the effects of the treatment.

The results of this experiment are given in Appendix VI and are summarized in Figs. 14 to 17 and Tables 11 to 14.

Generally, resistance to cold appeared to increase as the age of the egg increased. Initial mortality occurred after 67h with 3h old eggs, after 151h with 24h old eggs, after 227h with 48h old eggs and after 151h with 96h old eggs. However, these results are misleading. After 227h the 48h old eggs suffered 11.4% mortality while, after the same time period, the 96h old eggs had only 9.1% mortality. The mortality difference is even greater after 340 h exposure with 79.8% and 53.2% respectively for the 48h and 96h old eggs.

The increased resistance to cold is better illustrated when LD 50's and LD 95's are compared. Eggs 3h old had an LD 50 and an LD 95 of respectively 90.57 $\pm$ 2.32h and 118.85 $\pm$ 5.87h. These values for 24h old eggs were respectively 193.06 $\pm$ 5.09h and 252.93 $\pm$ 13.14h. Eggs aged 48h and 96h accrued 50% mortality after 271.64 $\pm$ 8.26h and 337.29 $\pm$ 17.52h and 95% mortality after 389.05 $\pm$ 17.82h and 633.87 $\pm$ 55.77h.

The F-values between error and treatment were highly significant. F-values of 568.84; 733.5; 445.7 and 811.8 were recorded respectively for 3h, 24h, 48h and 96h exposures. The variance between treatments

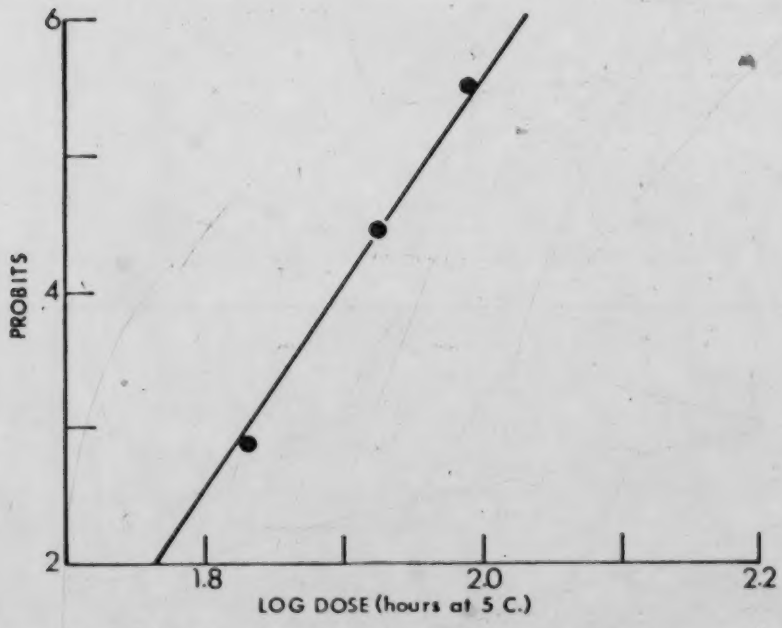



Fig. 14. Probit regression line showing the mortality, of various dosages of 5°C. on eggs 3 hours old.

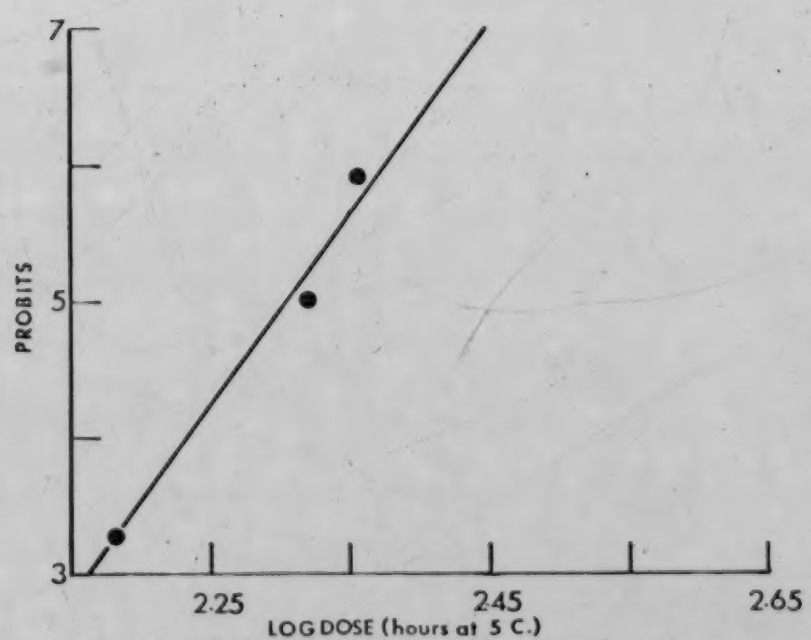



Fig. 15. Probit regression line showing the mortality, of various dosages of 5°C. on eggs 24 hours old.

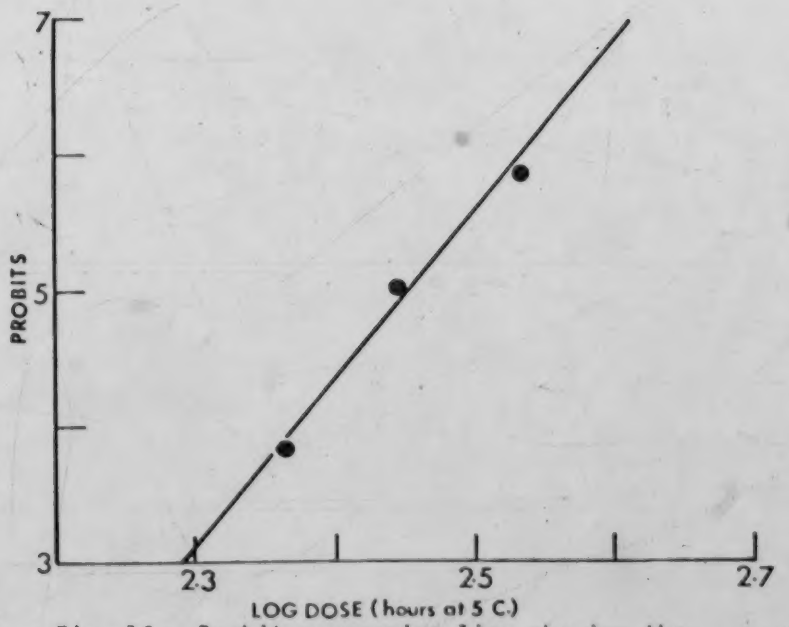



Fig. 16. Probit regression line showing the mortality, of various dosages of 5°C. on eggs 48 hours old.

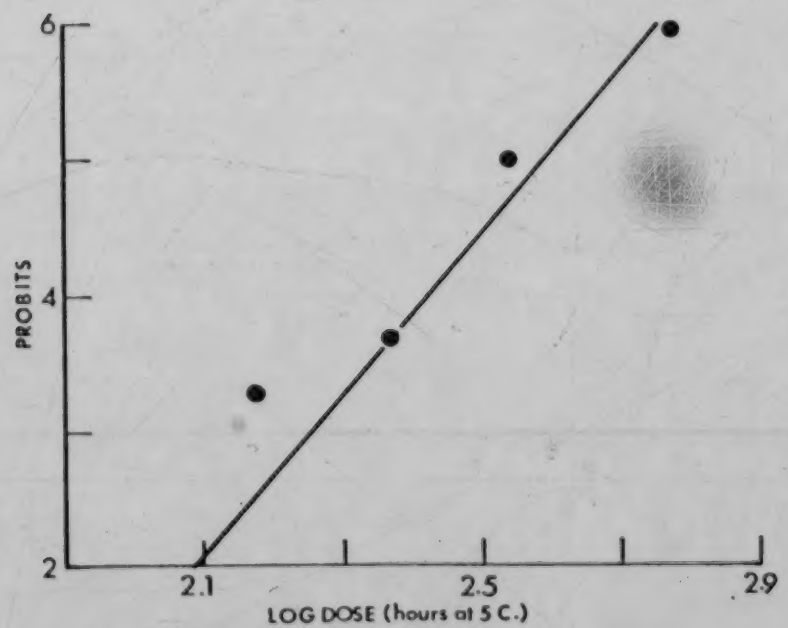



Fig. 17. Probit regression line showing the mortality, of various dosages of 5°C. on eggs 96 hours old.

TABLE 11 The Effect of Continuous Exposure to 5° C on Eggs of *M. configurata* Three Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 30 | 0 | |
| 120 | 45 | 0 | |
| 120 | 67.5 | 1.96 | 2.925 |
| 120 | 83 | 39.2 | 4.59 |
| 120 | 101 | 74 | 5.64 |
| 120 | 151 | 100 | |

Treatment F-value 568.84 (F at 1% level 3.85) with 5 DF

Between Treatments F-value 2.44 (F at 5% level 2.6) with 25 DF

Mean number of hours of exposure to 5° C required to produce 50% and 95% mortality are 90.57 $\pm$ 2.32 and 118.85 $\pm$ 5.87 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

TABLE 12 The Effect of Continuous Exposure to 5° C on Eggs of M. *configurata* 24 Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 30 | 0 | |
| 120 | 45 | 0 | |
| 120 | 67.5 | 0 | |
| 120 | 101 | 0 | |
| 120 | 151 | 4.46 | 3.300 |
| 120 | 189 | 50.14 | 5.0 |
| 120 | 227 | 82.087 | 5.919 |
| 120 | 340 | 100 | |

Treatment F-value 733.5 (F at 1% level 3.7) with 7 DF

Between Treatments 5.13 (F at 5% level 2.42) with 35 DF

Mean number of hours of exposure to 5° C required to produce 50% and 95% mortality are 193.06 $\pm$ 5.09 and 252.93 $\pm$ 13.14 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

TABLE 13 The Effect of Continuous Exposure to 5° C on Eggs of M. *configurata* 48 Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 30 | 0 | |
| 120 | 45 | 0 | |
| 120 | 67.5 | 0 | |
| 120 | 101 | 0 | |
| 120 | 151 | 0 | |
| 120 | 227 | 11.4 | 3.700 |
| 120 | 273 | 52.63 | 5.006 |
| 120 | 340 | 79.82 | 5.834 |
| 120 | 510 | 100 | |

Treatment F-value 445.7 (F at 1% level 3.51) with 8 DF

Between Treatments F-value 2.19 (F at 5% level 2.18) with 40 DF

Mean number of hours of exposure to 5° C required to produce 50% and 95% mortality are 271.64 $\pm$ 8.26 and 389.05 $\pm$ 17.82 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

TABLE 14 The Effect of Continuous Exposure to 5° C on Eggs of M. *congigurata* 96 Hours Old.

| No. of Individuals Tested | Length of Treatment in Hours | Net % Mortality | Emperical Probit |
|---|---|---|---|
| 120 | 30 | | |
| 120 | 45 | | |
| 120 | 67.5 | | |
| 120 | 101 | | |
| 120 | 151 | 4,75 | 3.3354 |
| 120 | 227 | 9.09 | 3.6654 |
| 120 | 340 | 53.2 | 5.0803 |
| 120 | 510 | 83.81 | 5.9463 |
| 120 | 765 | 100 | |

Treatment F-value 811.8(F at 1% level 3.51) with 8 DF

Between Treatments F-value 1.04 (F at 5% level 2.18) with 40 DF

Mean number of hours of exposure to 5° C required to produce 50% and 95% mortality are 337.29 ± 17.52 and 633.87 ± 55.77 respectively. Variance analysis showed that variation in percentage mortality was a function of treatment.

was significant only for the 24 h group.

## 6. THE EFFECTS OF ALTERNATING TEMPERATURE ON DEVELOPMENT

Alternating temperatures are used in biological research to determine effects that can not be ascertained using constant temperature alone. For example, under alternating temperatures, eggs of *O. fasciatus* can complete embryogenesis at a mean temperature several degrees lower than the lowest constant temperature at which complete embryogenesis can occur (Lin, et al, 1954).

During development, organisms are often subjected to extreme temperatures that would be fatal to them if they were maintained over long periods. Knowledge of the effects of alternating temperature on mortality and rate of development aids us in understanding naturally occurring temperature effects on development.

Two experiments were conducted on *M. configurata* to determine the effects of varying daily exposures to extreme temperatures on development rate. The first experiment was designed to determine the effects of daily exposure to 35° C on rate of development, the second had 5° C as the experimental temperature. In both experiments, the alternate temperature used was 20° C.

### 6.1 The effects of daily alternation between temperatures of 20°C and 35° C on eggs of different ages.

The main purpose of this experiment was to determine if eggs of the experimental population could withstand daily exposure to 35°C.

In Alberta, the ovipositional period of M. *configurata* extends from late June to mid-July so that its eggs would rarely be exposed to a temperature of 35° C. However, since M. *configurata* is partially bi-voltine ( i.e. it lacks either an obligatory or a facultative diapause in some members of its population), its eggs may be expected to show partial resistance to daily temperatures of 35° C.

The other purpose of this experiment was to determine if alternation of temperature influenced development rate. Alternation of a high temperature with a moderate temperature often results in a decrease in the development rate of an insect compared with its performance at a constant temperature equal to the mean of the alternating temperatures (Johnson, 1940).

### 6.1.1 MATERIALS AND METHODS

Eggs were collected as described in section 3.2 from a culture reared at 20 ± 0.5° C and approximately 60% RH. Collected eggs were divided into groups of 10 and each group was placed in a 1mm cap vial.

Four groups of 10 individuals were used at each of 13 experimental exposures (from 1 to 22.5h daily at 35° C; see Tables 15 to 18 for details). Eggs of four different ages (3, 24, 48 and 96h) were used to determine the effect of age on tolerance to 35° C.

Eggs from any one collection period were divided into as many

groups of 10 as was possible, and the resulting groups were subdivided into units of four. Each of these units were then given daily exposure to 35° C. One vial from each collection was left at 20 ± 0.5° C to determine fertility.

During exposure, the vials were placed on a platform in a desiccator. A saturated salt solution of $NH_4NO_3$ (Winston and Bates, 1960) was used to produce an RH of approximately 60%. First exposure to 35 ± 0.5° C was made immediately after the eggs had reached the desired age and was repeated at the same time each subsequent day until completion of the experiment. After exposure, the eggs were returned to 20 ± 0.5° C for the remainder of the day.

Eggs were observed daily until they reached the black head capsule stage at which time observations were made at two hour intervals. The number hatching and the total length of the development period for each egg was then recorded.

The mean hatching time for each exposure and the mean temperature for each group was then determined. This allowed a comparison to be made of mean development between eggs maintained at constant temperatures (section 5.1.1) and those maintained under alternating temperatures.

Fry and Surber's (1971) experiment on the effects of exposure to 35° C and 40% RH on eggs of *E. acrea* was used as a model.

### 6.1.2 *RESULTS*

Results of the various treatments are given in Appendix VII and

are summarized in Tables 15 to 18. T-tests were used to determine if the treatments had any significant effect on development time. In general, daily exposures of one hour did not result in significant change in development time. Daily exposures of 2.9 h or greater resulted in a significant change in development time regardless of the number of exposures.

Duncan's New Multiple Range Test was used to determine if individual treatments within each experimental group varied significantly from each other. The difference between exposures of one hour and 1.3 h daily to 35° C were insignificant in the 3 h old groups. In-significant differences were shown also between daily exposures of 4.8 and 6.2 h for both the 3 h and 24 h old groups. Eggs 48 hours old showed no significant difference in development time for daily treatments of 2.9, 3.7, and 4.8 hours, nor between treatments of 4.8 and 6.2 h and 1.7 and 2.2 h. Eggs 96 hours old showed no significant difference in development time for daily treatments of 1, 1.3, 1.7 or 2.2 h. No significant variation occurred between treatments of 3.7 and 4.8 or 8.0 and 10.4 h.

Variance analysis was also used to determine if the variation noted in development times was due to treatment. The resulting values showed that the treatments were highly significant in this.

A comparison between development times observed under constant temperature and under equivalent alternating temperatures showed that

TABLE 15 Effects of a Daily Exposure to 35° C on Development in Three Hour Old Eggs of *M. configurata*

| No. of Individuals Tested | Daily Exposure to 35° C (h) | Total Exposure to 35° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 6 | 132.514 ± 1.63 A* | ** |
| 40 | 1.3 | 7.8 | 132.375 ± 2.3 A | ** |
| 40 | 1.7 | 10.2 | 130.32 ± 1.65 | 0.005 |
| 40 | 2.2 | 13.2 | 129.33 ± 2.70 | 0.001 |
| 40 | 2.9 | 17.4 | 126.00 ± 2.27 | 0.001 |
| 40 | 3.7 | 20.4 | 124.82 ± 1.89 | 0.001 |
| 40 | 4.8 | 24 | 123.4 ± .458 B | 0.001 |
| 40 | 6.2 | 31 | 123.15 ± 2.64 B | 0.001 |
| 40 | 8.0 | 40.0 | 122.52 ± 2.82 | 0.001 |
| 40 | 10.4 | 52.0 | 121.33 ± 2.44 | 0.001 |

Treatment F-value 114.08 (F at 1% level 2.32) with 9 DF

*Means followed by the same letter signify that they are not significantly different (based on Duncan's New Multiple Range Test).

**Not significant at the 5% level using T-Tests. Variance analysis showed that the difference noticed in development times was a product of the various treatments.

TABLE 16 Effects of a Daily Exposure to 35° C on Development in 24 Hour Old Eggs of M. *configurata*

| No. of Individuals Tested | Daily Exposure to 35° C (h) | Total Exposure to 35° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 5 | 130.51 ± 1.88 | ** |
| 40 | 1.3 | 6 | 130.16 ± 2.15 | 0.05 |
| 40 | 1.7 | 7 | 128.43 ± 2.06 | 0.001 |
| 40 | 2.2 | 11 | 127.51 ± 1.59 | 0.001 |
| 40 | 2.9 | 14.5 | 125.12 ± 1.75 | 0.001 |
| 40 | 3.7 | 15 | 123.92 ± 1.58 | 0.001 |
| 40 | 4.8 | 16 | 121.55 ± 2.17 A* | 0.001 |
| 40 | 6.2 | 24.8 | 121.31 ± 1.95 A | 0.001 |
| 40 | 8.0 | 32 | 120.14 ± 1.63 | 0.001 |
| 40 | 10.4 | 40.8 | 119.33 ± 2.00 | 0.001 |
| 40 | 13.5 | 54.0 | 117.82 ± 1.90 | 0.001 |

Treatment F-value 174.89 (F at 1% level 2.32) with 10 DF

* means followed by the same letter signify that they are not significantly different (based on Duncan's New Multiple Range Test).

** not significant at the 5% level using T-Tests. Variance analysis showed that the difference noticed in development times was a product of the various treatments.

TABLE 17 Effects of a Daily Exposure to 35° C on Development in 48 Hour Old Eggs of *M. configurata*.

| No. of Individuals Tested | Daily Exposure to 35° C (h) | Total Exposure to 35° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 4 | 129.94 ± 1.39 | ** |
| 40 | 1.3 | 4.8 | 129.00 ± 1.63 | ** |
| 40 | 1.7 | 5.6 | 128.32 ± 1.80 A* | 0.05 |
| 40 | 2.2 | 8.8 | 128.15 ± 1.74 A | 0.05 |
| 40 | 2.9 | 11.6 | 126.06 ± 1.64 B | 0.001 |
| 40 | 3.7 | 12 | 125.78 ± 1.62 B | 0.001 |
| 40 | 4.8 | 19.2 | 125.5 ± 1.54 B C | 0.001 |
| 40 | 6.2 | 23.09 | 124.49 ± 1.28 C | 0.001 |
| 40 | 8.0 | 28.05 | 124.05 ± 1.37 | 0.001 |
| 40 | 10.4 | 34.7 | 123.53 ± 1.43 | 0.001 |
| 40 | 13.5 | 42.5 | 122.0 ± 1.30 | 0.001 |
| 40 | 17.5 | 51.5 | 119.27 ± 1.35 | 0.001 |

Treatment F-value 105.36 (F at 1% level 2.32) with 11 DF

*means followed by the same letter signify that they are not significantly different (based on Duncan's New Multiple Range Test).

**Not significant at the 5% level using T-Test.

Variance analysis showed that the difference noticed in development times was a product of the various treatments.

TABLE 18 Effects of a Daily Exposure to 35° C on Development in 96 Hour Old Eggs of M. *configurata*

| No. of Individuals Tested | Daily Exposure to 35° C (h) | Total Exposure to to 35° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 2 | 132.78 ± 1.10 A* | ** |
| 40 | 1.3 | 2.4 | 132.75 ± 1.32 A | ** |
| 40 | 1.7 | 3.4 | 132.58 ± 1.06 A | ** |
| 40 | 2.2 | 4.4 | 132.72 ± 1.84 A | ** |
| 40 | 2.9 | 5.8 | 132.058 ± 1.87 | 0.05 |
| 40 | 3.7 | 6.0 | 131.62 ± 1.82 B | 0.05 |
| 40 | 4.8 | 9.6 | 131.44 ± 1.62 B | 0.001 |
| 40 | 6.2 | 12.4 | 130.91 ± 1.66 | 0.001 |
| 40 | 8.0 | 16.0 | 130.00 ± 1.61 C | 0.001 |
| 40 | 10.4 | 18.96 | 129.74 ± 1.84 C | 0.001 |
| 40 | 13.5 | 21.29 | 128.56 ± 1.40 | 0.001 |
| 40 | 17.5 | 29.89 | 127.79 ± 1.53 | 0.001 |
| 40 | 22.5 | 28.89 | 126.39 ± 1.41 | 0.001 |

Treatment F-value 56.39 (F at 1% level 2.32) with 12 DF

* means followed by the same letter signify that they are not significantly different (based on Duncan's New Multiple Range Test).

** Not significant at the 5% level using T-Tests.

Variance analysis showed that the difference noticed in development times was a product of the various treatments.

development at a constant temperature usually occurred more rapidly than at an equivalent temperature produced by daily alternation between 35°C and 20°C., (Table 19).

My results were difficult to reconcile with those of Fry and Surber (1971). A single exposure to 35° C and 40% RH for 20 h resulted in a 1.6% hatch of salt marsh caterpillar eggs, whereas all eggs of M. *configurata* hatched that were given a similar exposure to 35° C and 60% RH for 22.8 h. However, three exposures of 16 h to 35° C and 40% RH resulted in 65.7% hatch for salt marsh caterpillar eggs while three similar exposures of 17.5 h to 35° C and 60% RH resulted in only 27.5% hatch for M. *configurata* eggs.

## 6.2 The Effects of Daily Alternation Between Temperatures of 5°C and 20°C on Eggs of Different Ages.

In the province of Alberta, M. *configurata* has been recorded from almost all rape growing areas extending from south of Lethbridge north to Keg River, a distance of almost 600 miles, (Philip, 1972). Over much of this range, temperatures during the ovipositional period (June to July) often fall to 5° C or lower (below the develomental-hatching threshold) for considerable parts of the day (Canadian Department of Transport Meteorlogical Records, 1971-1974). The primary purpose of this experiment was to determine the effects of a varying exposure to 5° C on development time of eggs. A secondary purpose was to determine if alternation of temperatures would produce

TABLE 19 A COMPARISON OF DEVELOPMENT RATE IN HOURS AT CONSTANT TEMPERATURE AND AT AN EQUIVALENT ALTERNATING TEMPERATURE (35° and 20° C) OF EGGS OF M. *configurata*.

| Temperature in C° | Development Time in Hours | |
|---|---|---|
| | Constant | Alternating |
| 20 | 135.14 | 132.16 |
| 22.5 | 118.8 | 121.55 |
| 25 | 100.92 | 119.33 |

an acceleration in egg development over those maintained at a constant equivalent temperature.

### 6.2.1 MATERIALS AND METHODS

Methods and materials used in this experiment were identical to those of the previous one (6.1) except that (1) daily exposure was to 5° C, (2) the saturated salt solution used to maintain 60% RH was $Na_2Cr_2O_7.H_2O$, and (3) 25 rather than 10 individuals were used in experiments involving three hour old eggs.

### 6.2.2 RESULTS

The effects of the various treatments are given in Appendix VIII and are summarized in Tables 20 to 23.

T - tests were used to determine the significance of the effect of treatment on development time. In all cases, the treatments produced a significant delay in development as compared with controls. Variance analysis was used to determine if the difference noted in development times was a function of treatment. In all cases, the difference noted between development times was highly significant at the 1% confidence level.

Duncan's New Multiple Range Test was used to determine if the individual treatments produced a significant difference in development times within individual test groups. In all cases, this test showed

TABLE 20 Effects of a Daily Exposure to 5° C on Development in Three Hour Old Eggs of *M. configurata*

| No. of Individuals Tested | Daily Exposure to 5° C (h) | Total exposure to 5° C (h) | Mean Development Time of combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 100 | 1.0 | 6.0 | 137.54 ± 1.99 | 0.001** |
| 100 | 1.3 | 7.8 | 138.76 ± 1.84 | 0.001 |
| 100 | 1.7 | 10.2 | 142.08 ± 2.11 | 0.001 |
| 100 | 2.2 | 13.2 | 141.12 ± 1.74 | 0.001 |
| 100 | 2.9 | 17.4 | 142.31 ± 1.83 | 0.001 |
| 100 | 3.7 | 25.9 | 156.51 ± 2.00 | 0.001 |
| 100 | 4.8 | 33.6 | 163.91 ± 1.34 | 0.001 |
| 100 | 6.2 | 49.6 | 180.79 ± 1.57 | 0.001 |
| 100 | 8.0 | 64.0 | 194.76 ± 1.74 | 0.001 |
| 100 | 10.4 | 102.5 | 233.92 ± 1.65 | 0.001 |
| 100 | 13.5 | 162.0 | 289.93 ± 2.11 | 0.001 |
| 100 | 17.5 | 332.5 | 454.20 ± 1.78 | 0.001 |

Treatment F-value 550 (F at 1% level 2.32) with 11 DF

** level of significance comparing mean development times of the combined replicates and the control group using T-Tests.

The difference in development times between treatments was analysed by variance analysis and found to be highly significant and, almost completely, a function of treatment.

TABLE 21 Effects of a Daily Exposure to 5° C on Development in 24 Hour Old Eggs of *M. configurata*

| No. of Individuals Tested | Daily Exposure to 5° C (h) | Total Exposure to 5° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 5 | 135.45 ± 0.997 | 0.001** |
| 40 | 1.3 | 6.5 | 138.50 ± 1.66 | 0.001 |
| 40 | 1.7 | 8.5 | 140.29 ± 1.78 | 0.001 |
| 40 | 2.2 | 11 | 141.16 ± 1.61 | 0.001 |
| 40 | 2.9 | 14.5 | 145.83 ± 1.54 | 0.001 |
| 40 | 3.7 | 22.2 | 151.25 ± 2.81 | 0.001 |
| 40 | 4.8 | 28.2 | 160.96 ± 1.35 | 0.001 |
| 40 | 6.2 | 37.2 | 173.07 ± 1.73 | 0.001 |
| 40 | 8.0 | 56 | 183.61 ± 1.74 | 0.001 |
| 40 | 10.4 | 93.6 | 230.69 ± 1.76 | 0.001 |
| 40 | 13.5 | 136 | 258.5 ± 1.34 | 0.001 |
| 40 | 17.5 | 262.5 | 387.22 ± 1.53 | 0.001 |

Treatment F-value 63,708 (F at 1% level 2.32) with 11 DF

** level of significance comparing mean development times of the combined replicates and the control group using T-Tests.

The difference in development time between treatments was analysed using variance analysis and found to be highly significant and, almost completely, a function of treatment.

TABLE 22 Effects of a Daily Exposure to 5° C on Development in 48 Hour Old Eggs of *M. configurata*.

| No. of Individuals Tested | Daily Exposure to 5° C (h) | Total Exposure to to 5° C (h) | Mean Development Time of Combined Replicates (h) | Level of Sig. bet. Check Hatching Time & Combined Rep. |
|---|---|---|---|---|
| 40 | 1 | 4 | 138.95 ± 1.90 | 0.001** |
| 40 | 1.3 | 5.2 | 140.38 ± 1.50 | 0.001 |
| 40 | 1.7 | 6.8 | 142.95 ± 2.01 | 0.001 |
| 40 | 2.2 | 8.8 | 144.82 ± 1.57 | 0.001 |
| 40 | 2.9 | 14.5 | 149.78 ± 1.64 | 0.001 |
| 40 | 3.7 | 18.5 | 155.19 ± 1.91 | 0.001 |
| 40 | 4.8 | 24 | 159.66 ± 1.49 | 0.001 |
| 40 | 6.2 | 31 | 165.94 ± 1.82 | 0.001 |
| 40 | 8.0 | 40 | 176.03 ± 1.46 | 0.001 |
| 40 | 10.4 | 62.4 | 194.72 ± 1.73 | 0.001 |
| 40 | 13.5 | 108 | 236.61 ± 1.84 | 0.001 |
| 40 | 17.5 | 210 | 339.41 ± 1.52 | 0.001 |

Treatment F-value 39,499 (F at 1% level 2.32) with 11 DF

**level of significance comparing mean development times of the combined replicates and the control group using T-Tests.

The difference in development times between treatments was analysed using variance analysis and was found to be highly significant and, almost completely, a function of treatment.

TABLE 23 Effects of a Daily Exposure to 5° C on Development in 96 Hour Old Eggs of *M. configurata*

| No. of Individuals Tested | Daily Exposure to 5° C (h) | Total Exposure to 5° C (h) | Mean Development Time of Combined Replicates (h) | Level of Significance between Check Hatching Time and Combined Replicates |
|---|---|---|---|---|
| 40 | 1 | 2 | 133.83 ± 1.68 | 0.001** |
| 40 | 1.3 | 2.6 | 135.91 ± 1.74 | 0.001 |
| 40 | 1.7 | 2.4 | 137.03 ± 1.67 A* | 0.001 |
| 40 | 2.2 | 4.4 | 137.18 ± 1.31 A | 0.001 |
| 40 | 2.9 | 5.6 | 138.67 ± 1.63 | 0.001 |
| 40 | 3.7 | 7.6 | 140.43 ± 2.71 | 0.001 |
| 40 | 4.8 | 9.6 | 141.84 ± 1.11 | 0.001 |
| 40 | 6.2 | 12.4 | 145.20 ± 1.95 | 0.001 |
| 40 | 8.0 | 24.0 | 156.65 ± 1.81 | 0.001 |
| 40 | 10.4 | 31.2 | 162.17 ± 1.81 | 0.001 |
| 40 | 13.5 | 54 | 184.94 ± 1.72 | 0.001 |
| 40 | 17.5 | 87.5 | 219.37 ± 1.66 | 0.001 |
| 40 | 22.5 | 308.5 | 435.33 ± 1.91 | 0.001 |

Treatment F-value 78,120 (F at 1% level 2.18) with 12 DF

* means followed by the same letter are not significantly different based on Duncan's New Multiple Range Test.

** level of significance comparing mean development times of the combined replicates and the control group using T-Tests.

The difference in development times between treatments was analysed using variance analysis and was found to be highly significant and, almost completely, a function of treatment.

that the treatments differed at the 5% confidence level.

When the rates of development of eggs under constant temperature and under its alternate temperature equivalent were compared those under the alternating temperatures developed more rapidly (Table 24).

TABLE 24 A COMPARISON OF DEVELOPMENT RATE IN HOURS AT CONSTANT TEMPERATURE AND AT AN EQUIVALENT ALTERNATING TEMPERATURE (5° and 20° C) OF EGGS OF M. *configurata*

| Temperature in C° | Development Time in Hours | |
|---|---|---|
| | Constant | Alternating |
| 17.5 | 220.71 | 156.5 |
| 15 | 252.32 | 194.76 |
| 12.5 | 462.65 | 387.22 |

# 7.0 DISCUSSION AND CONCLUSIONS

## 7.1 THE EGG

Eggs of M. *configurata*, in the population I studied, appear to be slightly larger (1.25mm) than those reported by Rempel (1951). This difference may be due to nutritional differences in the two moth populations, the newer improved varieties of rape being superior hosts.

King (1928) reported approximately 38 ribs on the egg; my results suggest about 36, ranging from 30-40. Structure of the outer and inner micropyle is similar to that described for *Amanthes c-nigrum* (Lepidoptera: Noctuidae) by Salkeld (1973).

### 7.1.2 METHODS OF COLLECTING AND HANDLING EGGS

M. *configurata* is an ideal subject for experiments requiring accurately timed eggs.

Other than preferences of temperature and darkness, the female appears to be devoid of any ovipositional prejudices. The most important characteristic of these eggs, is, that at approximately 20° C, fertilization does not occur until over 1/2 hour after oviposition (Rempel, 1951). This allows eggs to be transferred before much development has occurred.

### 7.1.3 *VIABILITY AND SIZE OF FIELD-DEPOSITED versus LABORATORY-DEPOSITED EGGS*

Eggs of *M. configurata* are very viable under favorable conditions, with an average hatch of over 96%. No increase in mortality over naturally occurring levels was noted if the eggs were handled carefully.

Laboratory-deposited egg clusters are significantly larger than their field-collected counterparts. This is probably due to the ideal ovipositional conditions of the laboratory. For example, the female does not have to expend energy in locating her host plant.

## 7.2 TEMPERATURE THRESHOLDS FOR EMBRYOGENSIS

The various temperature thresholds demonstrated for embryogensis in *M. configurata* show that its eggs are well adapted to the Alberta climatic conditions occurring at the time of oviposition. Some development occurs at temperatures as low as 2° C. Fully developed eggs can hatch at temperatures as low as 5° C (3.5° C below the developmental-hatching threshold). Eggs can develop completely and hatch at temperatures ranging from 8.5° C to 30° C. However mortality at the developmental-hatching threshold is high (77.8%). Normal temperatures (mean, maximum and minimum) for the ovipositional period only rarely fall outside the developmental range (Depart. of Transport Meteorlogical Records).

Similar high mortality at the developmental-hatching threshold has been demonstrated for *O. fasciatus* (Richards and Kolderie, 1957). It is probable that the larvae that hatched would have not reached maturity. Their hatching behavior showed the same anomalies as those reported by Lin, et al (1954) for nymphs of *O. fasciatus*. They found that even if these nymphs were transferred to ideal conditions, very few reached maturity. Larvae hatching under these conditions are extremely debilitated and have difficulty in locomotion. It is possible that these larvae have internal structural defects that doom them to an early death.

The upper developmental-hatching threshold for eggs of *M. configurata* (between 30 and 31.5° C) is comparable to that of eggs of the armyworm, *Pseudaletia unipuncta* (Howe) (Lepidoptera:Noctuidae) a serious pest of various crops in Canada (Guppy, 1969). He found that the rate of development for this species began to decrease when temperature was increased above 29° C. Eggs of *M. configurata* probably follow a similar course of development. The lack of data supporting this belief probably arises from experimental error. Sample sizes (50 individuals) may have been too small to reveal the small percentage of individuals that might have hatched at temperatures above 30 ° C. Also, the interval between 30° C and the next temperature (31.5° C) might have been too large. Mortality in insects increases rapidly as temperature increases above their optimum temperatures (Stinner et al, 1974). Even if complete development is curtailed by temperatures in excess of 30° C, the occurrence of these temperatures in Western Canada during the incubation period is rare and would have little effect on development.

## 7.3 THE EFFECTS OF CONSTANT TEMPERATURE AND RELATIVE HUMIDITY ON EMBRYOGENSIS

The rate of embryogenesis showed a strong linear relationship with temperature over the range of temperatures used in this experiment. Deviation from this relationship occurred only at temperatures of 10° C and lower. Complete development and hatching occurred at temperatures ranging from 8.5° C to 30° C.

Low humidity retarded development regardless of temperature. It is possible that eggs of M. *configurata* normally absorb from the air at least some portion of the water used during development. Extremely low humidity (i.e. 0%) would completely inhibit this absorbtion and force development to rely upon already present reserves, thus slowing down the rate of development.

Lower temperatures prolonged the exposure to low humidities and resulted in increased mortality. Possible causes for this are (1) death due to desiccation as the eggs had insufficient water to complete development (2) weakening of the larvae through water loss, making the act of hatching more difficult and (3) a hardening of the chorion caused by desiccation, inhibiting hatching, or a combination of these.

The fastest rate of development always occurred at 98% RH followed by 60% RH and 0% RH. This suggests that M. *configurata* absorbs water during development and that in some cases water may become the limiting factor in some part of embryogencis. Water absorbtion would be easiest in high humidity, decreasing as humidity declines.

### 7.3.1 THE RELATION BETWEEN CONSTANT TEMPERATURE AND 0%, 60% AND 90% RH AND THEIR EFFECTS ON DEVELOPMENTAL RATE

In general, low relative humidities retard the rate of development in an insect egg. I have shown this to be true too for eggs of *M. configurata* (section 6.1). Regardless of the temperature used, hatching always occurred first at 98% RH followed by 60% and 0%. Relative humidity appears to be more important in the lower temperature range (15° C to 8.5°C). At 0% RH, hatching was completely impaired by temperatures below 15° C. At the developmental-hatching threshold (8.5° C) hatching occurred only at 98% RH.

These results agree with those reported for other Lepidoptera (Ludwick and Anderson, 1942). The reasons for this response can only be speculated upon. The drying effect of low humidity could harden the chorion and make hatching more difficult. In addition, the larvae could be weakened, through water loss, this slowing down their development and delaying hatching.

I suspect that all three and possibly additional factors retard and prevent hatching. Evidence from my experiments suggest that both mechanical and physiological barriers retard and reduce hatching is that the number of individuals to eclose at each of the humidities used remained relatively constant regardless of temperature. Differences of eight to 10 hours occurred between 0% and 98% RH, and six to eight hours between 60% and 98%RH. This evidence, however, could also be used to support the premise that the larvae were weakened and thus took longer to hatch. There is also evidence suggesting a physiological delay. Eggs exposed to 0% RH always required longer time to develop brown pigmentation (see Section 3.1). This suggests that early stages of develop-

ment are impaired by lack of access to atmospheric moisture. In many insects, development is enhanced by absorption of atmospheric moisture through a hydropyle (Wigglesworth, 1965). Although examination of eggs of M. *configurata* failed to locate a similar structure, the numerous aeropyles may act in a similar capacity.

The experiments I conducted were not designed to determine the mechanism by which humidity affects development. Experiments using a greater range of humidities and tests of egg shell tensile strength would aid in determining whether physiological or mechanical barriers have a greater effect on mortality and rate of development.

### 7.3.2. THE EFFECT OF CONSTANT EXPOSURE TO A TEMPERATURE OF 35° C ON DEVELOPMENT OF EGGS OF DIFFERENT AGES

Constant exposure to 35° C produced approximately 50% mortality after 37 hours and 95% mortality after 60 hours regardless of egg age (Section 5.2). A comparison of the 50% and 95% mortality times shows that older eggs may show slightly more tolerance than their younger counterparts. However, the difference is slight and only much larger sample sizes and the use of a greater number of exposures will determine if the difference is significant.

Sensitivity to high temperature decreased in Silkworm eggs, *Bombyx mori* L. (Lepidoptera:Bombycidae), after meiosis but before the beginning of karyogamy (Ostryakova-Varshaver, 1958). The exact cause of death at high temperatures is unknown. Denaturing of proteins, a change in

metabolism allowing a build up of toxic materials, desiccation or starvation have all been suggested as contributing to heat death (Chapman, 1971). It is possible that older eggs, that is those 24, 48 and 96 hours old, might have shown greater resistance to heat if they had been kept at 30° C prior to testing at 35° C. *Drosophila sp.*, show considerable variation in survival time depending upon the temperature they are reared at. Rearing at 25° C more than doubled their survival time at 33.5° C compared with counterparts reared at 15° C (Chapman, 1971).

There appear to be two types of acclimation: a longlasting "developmental" and a transitory "physiological" acclimation (Bursell, 1974). The first of these is dependent upon the temperature at which the insect was raised prior to the treatment. Insects raised at a higher temperature require a longer exposure to a particular high temperature to produce mortality equal to that of their counterparts raised at a lower temperature. This acclimation appears to be permanent and is not affected by a return to lower temperatures. The second type, physiological, is readily reversible, its effectiveness apparently being a function of both the temperature and the length of exposure used prior to exposure of the insect to the experimental temperature (Bursell, 1974). For example in one insect Baldwin and Riordan (1956, in Bursell, 1974) found that the greatest amount of acclimation occurred after two hours and declined to insignificant levels in the next 12 hours.

*7.3.3 THE EFFECT OF CONSTANT EXPOSURE TO A TEMPERATURE OF 5° C ON DEVELOPMENT OF EGGS OF DIFFERENT AGES*

Constant exposure to 5° C had varying results (Section 5.3) Resistance to cold increased with increasing age of the embryo suggesting that early stages of embryogenesis are more sensitive. Thus, early stages may require daily exposure to favorable temperature before they can complete development (Lin, et al, 1954). Two day old cabbage looper eggs, *Trichoplusia ni* (Hübner) exposed to 11° C, showed increased mortality compared to that of one or three day old eggs (Kishaba and Henneberry, 1966).

Death of embryos at low temperature may result from histological abnormalities. Some embryos of *Bombus mori*, stored at low temperatures, showed many abnormalities; eg. the amniotic cavity was larger than normal and often broken in some individuals. Others showed incomplete dorsal closure, resulting in parts of the alimentary canal being left outside the body (Totani, 1960). From these examples it appears that constant low temperatures cause a disruption of metabolism and subsequent development of the embryo.

Another cause of death after continuous exposure to suboptimum temperature may be the lack of sufficient food reserves to allow for complete development once the eggs are returned to a favorable temperature (Richards, 1964). This does not appear to be the cause of death for three h old eggs of M. *configurata* as none of these showed any development of pigmentation (a sign that they had not developed significantly

at the end of treatment). As in the previous experiment (see 7.3.2.) different results would have occurred if the eggs were pre-conditioned prior to exposure to the experimental temperature.

## 7.4 THE EFFECTS OF ALTERNATING TEMPERATURE ON DEVELOPMENT

Alternating temperatures appeared to either increase or decrease development rate depending on the situation. Alternation between 5° C and 20° C seemed to accelerate development compared with that occurring at an equivalent constant temperature. Alternation between 20° C and 35° C seemed to decrease development rate.

### *7.4.1 THE EFFECT OF A DAILY ALTERNATION BETWEEN TEMPERATURES OF 20° C AND 35° C ON EGGS OF DIFFERENT AGES*

Eggs of M. *configurata* demonstrated an increasing tolerance to longer daily exposures to 35° C as they matured. Eggs two days old at the beginning of the treatments withstood longer daily exposures than did younger eggs. However, this does not mean that they showed a greater overall resistance to temperatures of 35° C. When the total time spent at 35° C is compared it shows that, regardless of age, no eggs hatched from any group receiving in excess of 55 hours. This data, combined with that of the experiment on constant exposure to 35° C, (Section 5.2) suggest that the effects of 35° C may be cumulative. If this is true, it is unlikely that any one stage of embryogenesis is the most susceptible.

Rather, it suggests that the whole of metabolism is disrupted, leading to a build up of toxic materials and cellular disorganization, either or both of these causing death.

The time required for complete development was greater under alternating than under constant temperature. This agrees with similar results recorded for the Japanese beetle, *P. japonica* (Ludwick, 1928), *D. melanogaster* Meigen (Ludwick and Cable, 1933) and various fruit flies (Messenger and Flitters, 1958). The reason for this is the sigmoidal function of the temperature and rate of development curves. Above the optimum temperature (30° C for *M. configurata*), rate of development begins to decline, this decline increasing rapidly as temperature increases. Thus, the actual rate of development at 35° C is probably similar to that occurring at 30° C or lower. This means that the time spent at 35° C is equivalent to an identical time spent at 30° C and that this should be calculated on this basis rather than on a hypothetical linear relationship supposedly existing between temperature and development. If the actual sigmoidal relationship is used, neither a retardation nor acceleration of development would appear to occur.

*M. configurata* reacts similarily to the saltmarsh caterpillar, *E. acrea*, when exposed to 35° C (Fry and Surber, 1971). This suggests that eggs of *M. configurata* are sufficiently tolerant to survive temperatures found in the Southwestern United States if the ovipositional period of *M. configurata* there is similar to that of *E. acrea*.

### 7.4.2 THE EFFECTS OF DAILY ALTERNATION BETWEEN TEMPERATURES OF 5° C AND 20° C ON EGGS OF DIFFERENT AGES

The effects of daily exposure to 5° C (Section 6.2) are more applicable to conditions found in Alberta. This experiment showed that eggs of M. *configurata* are well adjusted to Alberta climatic conditions. A temperature of 5° C, when alternated with a favorable temperature (such as 20° C), do not have an appreciable effect on mortality. The only instance of high mortality was when eggs were exposed to 5° C for more than 22 hours daily. In these, hatching occurred only in the 96 hour group even though development to the black head capsule stage occurred in all eggs tested. Failure to hatch of these eggs was probably because the larvae were debilitated by long exposure to 5° C. These results compare favorably with those of Lin et al (1954). They found that as little as one hour per day spent at a favorable temperature allowed complete development of eggs of *O. fasciatus*.

Time for development was shorter under alternating than under constant temperature. Similar results have been recorded for A. *orthogonia* and C. *auxiliaris* (Cook, 1927). There are two reasons for this apparent acceleration: 1) some development is taking place at 5° C (See section 4.0), and 2) due to the sigmoidal nature of the temperature-rate of development curve, lower temperatures (5 ° C) contributes less to total development than those above the mean (Johnson, 1940).

## 7.5 EVOLUTIONARY AND PRACTICAL CONSIDERATIONS

It is probable that M. *configurata* originated further south and has gradually expanded its range northward. This would explain the existance of the partial second generation which still occurs in Alberta. Eggs of M. *configurata* also show some resistance to temperatures that they are unlikely to be exposed to in Alberta.

Since the timing of larval surveys is critical, results of this study, hopefully, will be used to remove some of the guesswork. Even working with only mean, minimum and maximum temperatures it will be possible to estimate hatching with some accuracy. Proper timing of the survey will allow for implementation of a more efficient control program. Up until now, control was not usually implemented until the larvae had reached at least the fourth instar and caused considerable damage. Control measures directed against earlier instars will result in less damage occurring and in better control.

Further investigation should be conducted to determine if 30° C is actually the upper development-hatching threshold. Larvae which survived either constant high or low temperatures during embryogensis should be reared to adulthood to determine if any effects of this treatment appear in later life.

The effects of humidity should be studied in greater detail in an attempt to determine the mechanism or mechanisms by which development is affected. A possible starting point would be to determine if eggs of M. *configurata* absorb moisture and if so to find out whether it is an active or a passive process.

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# 9. APPENDICES

## QUANTITATIVE CHARACTERISTICS OF EGGS OF M. *configurata*

Appendix I

| Number of Ribs | Height in u | Width in u |
|---|---|---|
| 30 33 36 34 35 | 450 416 400 | 566 550 583 566 |
| 35 35 35 32 35 | 370 383 370 | 583 550 550 550 |
| 38 35 35 36 35 | 433 350 400 | 566 600 533 550 |
| 32 33 35 35 36 | 400 416 450 | 600 583 566 583 |
| 36 37 36 38 38 | 433 370 370 | 566 612 566 600 |
| 35 36 37 37 36 | 400 400 450 | 612 583 500 550 |
| 38 38 35 35 35 | 416 416 450 | 600 566 583 583 |
| 38 40 36 36 40 | 433 433 433 | 583 566 633 633 |
| 39 | 400 | 583 583 |

| Number of Micropyles | Number of Primary Cells |
|---|---|
| 5 5 5 5 5 5 3 4 3 4 | 11 10 11 10 11 10 11 10 11 11 |
| 4 3 5 4 4 4 4 4 4 5 | 11 11 11 11 10 11 12 12 11 10 |
| 5 5 6 4 5 5 4 5 5 5 | 10 11 11 11 11 12 11 11 11 11 |
| 5 5 4 5 3 5 6 4 5 5 | 12 14 12 11 12 10 10 10 11 13 |
| 4 4 5 5 5 5 5 5 5 4 | 12 13 11 11 13 10 10 10 11 10 |
| | 12 12 12 13 12 11 11 11 14 12 |
| | 11 10 12 15 14 12 12 12 14 13 |
| | 12 15 12 11 13 11 11 14 10 12 |
| | 14 11 11 11 14 12 13 13 12 14 |
| | 14 |

# THE DEVELOPMENTAL-HATCHING THRESHOLD FOR M. *CONFIGURATA* AND THE EFFECTS OF RELATIVE HUMIDITY ON IT

Appendix II

| Temperature in ° C | Numbers Hatching* | | |
|---|---|---|---|
| | 0% R.H. | 60% R.H. | 98% R.H. |
| 6.5 | 0 0 0 | 0 0 0 | 0 0 0 |
| 7.5 | 0 0 0 | 0 0 0 | 0 0 0 |
| 8.5 | 0 0 0 | 0 0 0 | 6 8 6 |

| Temperature in ° C | Numbers Reaching Black Head Capsule Stage | | |
|---|---|---|---|
| | 0% R.H. | 60% R.H. | 98% R.H. |
| 6.5 | 0 0 0 | 0 0 0 | 0 0 0 |
| 7.5 | 0 0 0 | 0 0 0 | 4 6 5 |
| 8.5 | 0 0 0 | 4 3 5 | 9 11 12 |

| | Numbers Hatching in Checks * | % Hatch |
|---|---|---|
| Day 1 | 27 | 90 |
| Day 2 | 29 | 96.6 |
| Day 3 | 29 | 96.6 |
| | Mean | 94.444 |

*maximum hatch possible in each replicate is 30

THE HIGH TEMPERATURE-DEVELOPMENTAL THRESHOLD FOR EGGS OF *M. configurata* AND THE EFFECTS OF RELATIVE HUMIDITY ON IT

Appendix III

| Temperature | Numbers Hatching* | | |
|---|---|---|---|
| in ° C | 0% R.H. | 60% R.H. | 98% R.H. |
| 30 | 28 28 30 | 28 28 30 | 28 28 30 |
| 31.5 | 0 0 0 | 0 0 0 | 0 0 0 |
| 32.5 | 0 0 0 | 0 0 0 | 0 0 0 |
| 33.5 | 0 0 0 | 0 0 0 | 0 0 0 |

*maximum hatch possible for each replicate is 30

THE EFFECT OF RELATIVE HUMIDITY ON DEVELOPMENT RATE IN EGGS OF M. *CONFIGURATA*.

THE EFFECT OF 0 PERCENT RELATIVE HUMIDITY ON DEVELOPMENTAL RATE

Appendix IV

15 ° C

| Time in Hours | Numbers Hatching* | | |
|---|---|---|---|
| 258 | 5 | 5 | 8 |
| 260 | 5 | 3 | 2 |
| 262 | 4 | 4 | 4 |

17.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 224 | 6 | 2 | 4 |
| 226 | 10 | 14 | 14 |
| 228 | 4 | 6 | 2 |

20 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 141 | 6 | 2 | 4 |
| 143 | 12 | 12 | 10 |
| 145 | 10 | 8 | 8 |

22.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 122 | 2 | 2 | 2 |
| 124 | 11 | 10 | 13 |
| 126 | 17 | 18 | 15 |

25 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 106 | 5 | 4 | 7 |
| 108 | 7 | 12 | 7 |
| 110 | 14 | 12 | 12 |

27.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 97 | 4 | 4 | 6 |
| 99 | 7 | 10 | 7 |
| 101 | 15 | 16 | 15 |

30° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 90 | 8 | 10 | 8 |
| 92 | 12 | 10 | 16 |
| 94 | 8 | 8 | 6 |

*maximum hatch possible in each replicate is 30

Appendix IV (Cont.)

THE EFFECT OF 60 PERCENT RELATIVE HUMIDITY ON DEVELOPMENT RATE

| 10 ° C | | | | 12.5 ° C | | | |
|---|---|---|---|---|---|---|---|
| Time in Hours | Numbers | Hatching* | | Time in Hours | Numbers | Hatching | |
| 572 | 4 | 2 | 0 | 460 | 6 | 2 | 2 |
| 574 | 4 | 4 | 2 | 462 | 8 | 14 | 12 |
| 576 | 8 | 4 | 4 | 464 | 14 | 10 | 14 |
| 578 | 0 | 4 | 6 | | | | |

| 15 ° C | | | | 17.5 ° C | | | |
|---|---|---|---|---|---|---|---|
| Time in Hours | Numbers | Hatching | | Time in Hours | Numbers | Hatching | |
| 258 | 2 | 5 | 7 | 224 | 4 | 2 | 4 |
| 260 | 18 | 17 | 11 | 226 | 9 | 12 | 13 |
| 262 | 10 | 8 | 10 | 228 | 15 | 14 | 11 |

| 20 ° C | | | | 22.5 ° C | | | |
|---|---|---|---|---|---|---|---|
| Time in Hours | Numbers | Hatching | | Time in Hours | Numbers | Hatching | |
| 133 | 7 | 6 | 3 | 116 | 2 | 4 | 6 |
| 135 | 10 | 11 | 13 | 118 | 10 | 10 | 10 |
| 137 | 13 | 11 | 12 | 120 | 18 | 16 | 14 |

*maximum hatch possible in each replicate is 30

Appendix IV (Cont.)

25 ° C

| Time in Hours | Numbers | Hatching | |
|---|---|---|---|
| 98 | 6 | 6 | 4 |
| 100 | 6 | 4 | 10 |
| 102 | 13 | 16 | 13 |

27.5 ° C

| Time in Hours | Numbers | Hatching | |
|---|---|---|---|
| 89 | 4 | 10 | 4 |
| 91 | 26 | 1 | 21 |
| 93 | 0 | 14 | 5 |

30 ° C

| Time in Hours | Numbers | Hatching | |
|---|---|---|---|
| 83 | 9 | 11 | 10 |
| 85 | 11 | 13 | 10 |
| 87 | 8 | 4 | 6 |

Appendix IV (Cont.)

THE EFFECT OF 98 PERCENT RELATIVE HUMIDITY ON DEVELOPMENT RATE

| 8.5 ° C | | | | 10 ° C | | | |
|---|---|---|---|---|---|---|---|
| Time in Hours | Numbers | Hatching* | | Time in Hours | Numbers | Hatching | |
| 877 | 2 | 0 | 0 | 560 | 4 | 4 | 0 |
| 878 | 0 | 1 | 3 | 562 | 6 | 0 | 4 |
| 879 | 2 | 0 | 0 | 564 | 1 | 7 | 4 |
| 880 | 0 | 6 | 0 | 566 | 9 | 1 | 4 |
| 881 | 2 | 0 | 0 | 568 | 0 | 0 | 2 |
| 882 | 0 | 1 | 3 | 570 | 0 | 0 | 8 |
| 12.5 ° C | | | | 15 ° C | | | |
| Time in Hours | Numbers | Hatching | | Time in Hours | Numbers | Hatching | |
| 456 | 8 | 7 | 9 | 248 | 9 | 9 | 10 |
| 458 | 6 | 5 | 7 | 250 | 9 | 11 | 12 |
| 460 | 12 | 16 | 12 | 252 | 12 | 10 | 8 |

* maximum hatch possible in each replicate is 30

Appendix IV (Cont.)

17.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 216 | 2 | 2 | 2 |
| 218 | 9 | 12 | 13 |
| 220 | 15 | 14 | 13 |

20 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 131 | 8 | 6 | 6 |
| 133 | 13 | 11 | 10 |
| 135 | 9 | 13 | 14 |

22.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 114 | 3 | 6 | 3 |
| 116 | 12 | 9 | 11 |
| 118 | 15 | 15 | 16 |

25 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 96 | 4 | 6 | 4 |
| 98 | 13 | 7 | 12 |
| 100 | 11 | 15 | 14 |

27.5 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 87 | 3 | 9 | 4 |
| 89 | 27 | 8 | 21 |
| 91 | 0 | 5 | 5 |
| 93 | 0 | 8 | 0 |

30 ° C

| Time in Hours | Numbers Hatching | | |
|---|---|---|---|
| 80 | 8 | 9 | 9 |
| 82 | 11 | 12 | 15 |
| 84 | 9 | 7 | 6 |

* maximum hatch possible in each replicate is 30

THE EFFECT OF CONSTANT EXPOSURE TO 35 ° C ON DEVELOPMENT OF EGGS OF *M. CONFIGURATA* OF DIFFERENT AGES

Appendix V

CONSTANT EXPOSURE TO 35 ° C. on 3 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching* | | | | | |
|---|---|---|---|---|---|---|
| 0 | 18 | | | 20 | | |
| 13 | 17 | 18 | 16 | 20 | 20 | 20 |
| 20 | 18 | 17 | 18 | 20 | 20 | 18 |
| 30 | 13 | 12 | 10 | 12 | 11 | 11 |
| 45 | 3 | 2 | 3 | 3 | 3 | 2 |
| 67.5 | 0 | 0 | 0 | 0 | 0 | 0 |

CONSTANT EXPOSURE TO 35 ° C. on 24 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching | | | | | |
|---|---|---|---|---|---|---|
| 0 | 20 | 19 | | 19 | | |
| 13 | 18 | 18 | 20 | 19 | 19 | 19 |
| 20 | 18 | 18 | 19 | 18 | 18 | 19 |
| 30 | 9 | 13 | 13 | 9 | 10 | 9 |
| 45 | 3 | 2 | 2 | 3 | 3 | 4 |
| 67.5 | 0 | 0 | 0 | 0 | 0 | 0 |

* maximum hatch possible in each replicate is 20

Appendix V (Cont.)

CONSTANT EXPOSURE TO 35 ° C. on 48 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching | | | | | |
|---|---|---|---|---|---|---|
| 0 | 20 | | | 20 | | |
| 13 | 20 | 20 | 20 | 20 | 20 | 20 |
| 20 | 18 | 20 | 20 | 20 | 20 | 20 |
| 30 | 10 | 10 | 14 | 11 | 11 | 11 |
| 45 | 3 | 3 | 2 | 3 | 2 | 2 |
| 67.5 | 0 | 0 | 0 | 0 | 0 | 0 |

CONSTANT EXPOSURE TO 35 ° C. on 96 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching | | | | | |
|---|---|---|---|---|---|---|
| 0 | 20 | | | 18 | | |
| 13 | 18 | 19 | 18 | 17 | 18 | 19 |
| 20 | 20 | 19 | 18 | 19 | 19 | 19 |
| 30 | 9 | 10 | 13 | 13 | 12 | 12 |
| 45 | 5 | 4 | 4 | 5 | 4 | 3 |
| 67.5 | 0 | 0 | 0 | 0 | 0 | 0 |

# THE EFFECT OF CONSTANT EXPOSURE TO 5 ° C ON DEVELOPMENT OF EGGS OF *M. CONFIGURATA* OF DIFFERENT AGES

Appendix VI

## CONSTANT EXPOSURE TO 5 ° C. on 3 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching* | | | | | |
|---|---|---|---|---|---|---|
| 0 | 20 | 20 | 20 | 19 | 20 | 19 |
| 30 | 20 | 20 | 19 | 20 | 19 | 19 |
| 45 | 18 | 19 | 18 | 19 | 19 | 20 |
| 67.5 | 17 | 18 | 18 | 16 | 17 | 18 |
| 83.7 | 10 | 13 | 11 | 13 | 10 | 14 |
| 101.25 | 4 | 6 | 5 | 4 | 4 | 5 |
| 151.25 | 0 | 0 | 0 | 0 | 0 | 0 |

## CONSTANT EXPOSURE TO 5 ° C on 24 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching | | | | | |
|---|---|---|---|---|---|---|
| 0 | 19 | | 20 | 19 | | |
| 30 | 20 | 20 | 20 | 19 | 18 | 18 |
| 45 | 20 | 20 | 20 | 19 | 19 | 19 |
| 67.5 | 20 | 20 | 17 | 19 | 18 | 18 |
| 101.25 | 20 | 20 | 18 | 18 | 18 | 19 |
| 151.25 | 19 | 20 | 17 | 19 | 18 | 18 |
| 189.0 | 10 | 8 | 9 | 9 | 8 | 9 |
| 227.0 | 4 | 3 | 2 | 4 | 3 | 3 |
| 340.0 | 0 | 0 | 0 | 0 | 0 | 0 |

* maximum hatch possible in each replicate is 20

Appendix VI (Cont.)

CONSTANT EXPOSURE TO 5 ° C. on 48 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching* | | | | | |
|---|---|---|---|---|---|---|
| 0 | 20 | 20 | | 20 | | |
| 30 | 18 | 20 | 19 | 20 | 20 | 20 |
| 45 | 18 | 19 | 18 | 20 | 19 | 20 |
| 67.5 | 20 | 20 | 20 | 20 | 19 | 19 |
| 101.25 | 20 | 20 | 20 | 19 | 18 | 20 |
| 151.25 | 20 | 20 | 20 | 19 | 19 | 20 |
| 227.0 | 18 | 18 | 17 | 17 | 15 | 16 |
| 173.5 | 6 | 9 | 9 | 11 | 8 | 11 |
| 340.0 | 4 | 4 | 3 | 4 | 3 | 5 |
| 510.0 | 0 | 0 | 0 | 0 | 0 | 0 |

CONSTANT EXPOSURE TO 5 ° C. on 96 HOUR OLD EGGS

| Exposure in Hours | Numbers Hatching | | | | | |
|---|---|---|---|---|---|---|
| 0 | 19 | | 20 | 19 | 19 | |
| 30 | 20 | 19 | 20 | 20 | 19 | 20 |
| 45 | 20 | 20 | 19 | 18 | 19 | 20 |
| 67.5 | 20 | 20 | 20 | 19 | 19 | 18 |
| 101.25 | 20 | 20 | 20 | 19 | 19 | 19 |
| 151.25 | 19 | 18 | 16 | 18 | 18 | 18 |
| 227.0 | 18 | 17 | 17 | 18 | 17 | 18 |
| 340.0 | 7 | 8 | 7 | 8 | 8 | 6 |
| 510.0 | 2 | 1 | 2 | 3 | 2 | 1 |
| 765.0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Maximum hatch possible in each replicate is 20

# THE EFFECT OF A DAILY EXPOSURE TO 35° C ON EGGS OF *M. CONFIGURATA* OF DIFFERENT AGES

Appendix VII

## DAILY EXPOSURE TO 35° C OF 3 HOUR OLD EGGS

CHECK 1-1.7 h

| Time in Hours | Numbers Hatching* |
|---|---|
| 130 | 2 |
| 132 | 1 |
| 134 | 4 |
| 136 | 3 |

CHECK 2.2 h

| Time in Hours | Numbers Hatching |
|---|---|
| 132 | 3 |
| 134 | 3 |
| 136 | 3 |

CHECK 2.9-10.4 h

| Time in Hours | Numbers Hatching |
|---|---|
| 132 | 2 |
| 134 | 1 |
| 136 | 2 |
| 138 | 4 |

1 HOUR DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 2 3 1 2 |
| 132 | 3 2 2 3 |
| 134 | 4 4 5 4 |

1.3 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 1 2 1 2 |
| 132 | 4 2 4 2 |
| 134 | 1 1 2 4 |
| 136 | 2 2 0 |

1.7 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 2 3 3 2 |
| 130 | 2 3 4 3 |
| 132 | 5 4 3 4 |

2.2 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 3 1 3 1 |
| 130 | 3 3 3 2 |
| 132 | 1 2 1 3 |
| 134 | 1 1 1 2 |
| 136 | 0 2 1 2 |

2.9 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 123 | 3 2 2 1 |
| 125 | 1 2 3 4 |
| 127 | 2 2 2 1 |
| 129 | 3 2 2 2 |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

| 3.7 HOURS DAILY | | 4.8 HOURS DAILY | | 6.2 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching* | Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 121 | 1 0 1 0 | 118 | 1 0 0 1 | 118 | 1 0 1 0 |
| 123 | 2 2 2 2 | 120 | 1 2 3 2 | 120 | 1 3 1 4 |
| 125 | 3 4 2 5 | 122 | 3 2 2 2 | 122 | 1 1 3 2 |
| 127 | 2 2 3 1 | 124 | 1 2 0 1 | 124 | 3 2 2 1 |
| | | 126 | 2 4 5 4 | 126 | 4 4 3 3 |
| | | 128 | 2 0 0 0 | | |

| 8.0 HOURS DAILY | | 10.4 HOURS DAILY | |
|---|---|---|---|
| Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 118 | 0 1 0 1 | 116 | 1 1 0 1 |
| 120 | 4 3 4 3 | 118 | 1 1 1 1 |
| 122 | 2 1 1 2 | 120 | 2 1 2 2 |
| 124 | 1 3 4 1 | 122 | 4 5 3 3 |
| 126 | 3 1 1 2 | 124 | 1 2 4 3 |

* maximum hatch possible in each replicate is 10

DAILY EXPOSURE TO 35° C OF 24 HOUR OLD EGGS

Appendix VII (Cont.)

CHECK 1-2.2 h

| Time in Hours | Numbers Hatching* |
|---|---|
| 128 | 3 |
| 130 | 1 |
| 132 | 3 |
| 134 | 1 |

CHECK 2.9-13.5 h

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 4 |
| 132 | 4 |
| 134 | 1 |
| 136 | 1 |

1 HOUR DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 4 3 2 1 |
| 130 | 2 3 3 4 |
| 132 | 4 3 5 2 |
| 134 | 0 0 0 3 |

1.3 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 4 3 4 4 |
| 130 | 2 4 2 2 |
| 132 | 2 1 2 3 |
| 134 | 1 1 2 1 |

1.7 HOURS DAILY

| Time Hours | Numbers Hatching |
|---|---|
| 126 | 4 2 3 1 |
| 128 | 3 1 4 7 |
| 130 | 2 2 1 1 |
| 132 | 0 4 1 1 |

2.2 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 126 | 3 5 5 4 |
| 128 | 3 4 3 2 |
| 130 | 2 1 2 3 |

2.9 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 122 | 2 0 0 0 |
| 124 | 3 6 2 5 |
| 126 | 2 2 3 1 |
| 128 | 1 3 2 1 |

3.7 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 122 | 1 2 3 2 |
| 124 | 2 2 4 3 |
| 126 | 3 2 1 1 |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

| 4.8 HOURS DAILY | | 6.2 HOURS DAILY | | 8.0 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching* | Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 118 | 0 1 1 2 | 118 | 1 0 0 2 | 118 | 0 3 3 1 |
| 120 | 3 4 1 1 | 120 | 3 4 1 1 | 120 | 4 3 3 3 |
| 122 | 2 1 3 3 | 122 | 1 1 3 3 | 122 | 2 1 0 4 |
| 124 | 1 2 1 1 | 124 | 1 2 3 0 | 124 | 1 0 0 0 |

| 10.4 HOURS DAILY | | 13.5 HOURS DAILY | |
|---|---|---|---|
| Time in Hours | Numbers Hatching | Time Hours | Numbers Hatching |
| 116 | 0 0 1 2 | 114 | 1 1 0 0 |
| 118 | 3 4 2 1 | 116 | 2 1 1 1 |
| 120 | 1 1 2 3 | 118 | 2 2 2 3 |
| 122 | 3 1 1 2 | 120 | 0 1 4 2 |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

DAILY EXPOSURE TO 35° C OF 48 HOUR OLD EGGS

CHECK 1 h

| Time in Hours | Numbers Hatching* |
|---|---|
| 128 | 2 |
| 130 | 4 |
| 132 | 3 |

CHECK 1.3-17.5 h

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 3 |
| 130 | 4 |
| 132 | 1 |
| 134 | 1 |

1.0 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 3 3 1 1 |
| 130 | 4 6 2 5 |
| 132 | 1 1 3 2 |

1.3 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 127 | 3 1 3 0 |
| 129 | 6 8 2 3 |
| 131 | 1 0 2 3 |
| 133 | 0 0 2 3 |

1.7 HOURS DAILY

| Time Hours | Numbers Hatching |
|---|---|
| 127 | 2 1 2 5 |
| 129 | 3 5 4 1 |
| 131 | 4 2 4 2 |

2.2 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 126 | 2 2 4 7 |
| 128 | 5 4 3 2 |
| 130 | 2 3 3 1 |
| 132 | 1 0 0 0 |

2.9 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 124 | 3 3 1 1 |
| 126 | 4 6 2 5 |
| 128 | 1 1 2 1 |
| 130 | 0 0 1 1 |

3.7 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 124 | 4 4 3 3 |
| 126 | 5 1 4 3 |
| 128 | 1 5 2 2 |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

| 4.8 HOURS DAILY | | 6.2 HOURS DAILY | | 8.0 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching* | Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 124 | 4 4 4 4 | 122 | 0 3 1 0 | 122 | 1 3 2 2 |
| 126 | 3 2 4 4 | 124 | 5 4 5 6 | 124 | 4 5 6 5 |
| 128 | 2 1 2 2 | 126 | 4 2 1 2 | 126 | 4 3 3 3 |

| 10.4 HOURS DAILY | | 13.5 HOURS DAILY | | 17.5 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 122 | 3 4 4 4 | 120 | 2 2 1 2 | 118 | 1 1 2 1 |
| 124 | 4 4 4 5 | 122 | 6 4 6 4 | 120 | 1 1 1 1 |
| 126 | 2 2 1 0 | 124 | 0 3 1 3 | 122 | 0 1 1 0 |
| 128 | 0 0 1 0 | | | | |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

DAILY EXPOSURE TO 35° C OF 96 HOUR OLD EGGS

CHECK 1-22.5 h

| Time in Hours | Numbers Hatching* |
|---|---|
| 132 | 4 |
| 134 | 4 |
| 136 | 1 |

1.0 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 0 0 1 0 |
| 132 | 5 4 6 5 |
| 134 | 5 6 1 3 |

1.3 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 0 2 0 1 |
| 132 | 4 3 4 3 |
| 134 | 3 5 4 3 |

1.7 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 1 0 0 0 |
| 132 | 6 6 4 3 |
| 134 | 1 2 4 4 |

2.2 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 130 | 2 2 3 3 |
| 132 | 2 2 2 1 |
| 134 | 2 3 2 3 |
| 136 | 2 2 1 1 |

2.9 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 1 0 0 1 |
| 130 | 1 1 4 2 |
| 132 | 3 5 1 2 |
| 134 | 3 3 4 3 |

3.7 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 2 0 0 1 |
| 130 | 2 2 2 2 |
| 132 | 5 4 3 5 |
| 134 | 0 4 3 2 |

4.8 HOURS DAILY

| Time in Hours | Numbers Hatching |
|---|---|
| 128 | 0 0 0 1 |
| 130 | 3 3 6 1 |
| 132 | 3 4 1 4 |
| 134 | 1 2 2 1 |

* maximum hatch possible in each replicate is 10

Appendix VII (Cont.)

| 6.2 HOURS DAILY | | 8.0 HOURS DAILY | | 10.4 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching* | Time in Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 128 | 0 2 1 0 | 126 | 0 0 0 1 | 126 | 0 0 0 1 |
| 130 | 4 5 4 3 | 128 | 1 3 2 1 | 128 | 2 3 3 4 |
| 132 | 2 1 3 4 | 130 | 4 3 4 4 | 130 | 3 2 2 3 |
| 134 | 2 1 1 0 | 132 | 1 3 2 3 | 132 | 2 2 3 1 |

| 13.5 HOURS DAILY | | 17.5 HOURS DAILY | | 22.5 HOURS DAILY | |
|---|---|---|---|---|---|
| Time in Hours | Numbers Hatching | Timein Hours | Numbers Hatching | Time in Hours | Numbers Hatching |
| 126 | 1 2 1 0 | 126 | 3 3 3 4 | 124 | 1 2 1 1 |
| 128 | 4 5 5 5 | 128 | 4 3 6 3 | 126 | 4 3 5 3 |
| 130 | 4 2 3 3 | 130 | 3 2 1 3 | 128 | 2 2 3 4 |
| 132 | 0 0 0 1 | | | | |

* maximum hatch possible in each replicate is 10

# THE EFFECT OF A DAILY EXPOSURE TO 5° C ON EGGS OF M. *CONFIGURATA* OF DIFFERENT AGES

Appendix VIII

## DAILY EXPOSURE TO 5° C of 3 HOUR OLD EGGS

### 1 HOUR DAILY

| Time in hours | no. hatching* |
|---|---|
| 134 | 2 3 3 4 |
| 136 | 6 7 7 7 |
| 138 | 8 8 8 9 |
| 140 | 9 7 7 5 |

### CHECK

| Time in hours | no. hatching |
|---|---|
| 128 | 5 |
| 130 | 4 |
| 132 | 13 |
| 134 | 3 |

### 1.3 HOURS DAILY

| Time in hours | no. hatching |
|---|---|
| 134 | 1 0 0 0 |
| 136 | 5 3 3 2 |
| 138 | 6 11 13 14 |
| 140 | 8 9 6 7 |
| 142 | 3 1 3 2 |

### CHECK

| Time in hours | no. hatching |
|---|---|
| 128 | 8 |
| 130 | 4 |
| 132 | 8 |
| 134 | 5 |

### 1.6 HOURS DAILY

| Time in hours | no. hatching |
|---|---|
| 138 | 1 1 0 1 |
| 140 | 7 8 8 6 |
| 142 | 9 9 9 8 |
| 144 | 3 3 4 5 |
| 146 | 3 3 3 3 |

### CHECK

| Time in hours | no. hatching |
|---|---|
| 128 | 8 |
| 130 | 5 |
| 132 | 7 |
| 134 | 5 |

* maximum hatch possible in each replicate is 25

Appendix VIII (Cont.)

2.1 HOURS DAILY

| Time in Hours | no. hatching* | | | |
|---|---|---|---|---|
| 139 | 1 | 2 | 3 | 1 |
| 141 | 8 | 8 | 13 | 7 |
| 143 | 8 | 10 | 4 | 7 |
| 145 | 5 | 3 | 3 | 6 |

2.9 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 139 | 3 | 2 | 2 | 4 |
| 141 | 8 | 5 | 6 | 8 |
| 143 | 8 | 11 | 9 | 10 |
| 145 | 5 | 5 | 5 | 2 |

3.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 154 | 7 | 7 | 4 | 3 |
| 156 | 9 | 11 | 9 | 8 |
| 158 | 3 | 2 | 5 | 5 |
| 160 | 3 | 3 | 3 | 5 |

4.8 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 162 | 5 | 6 | 6 | 6 |
| 164 | 15 | 12 | 12 | 13 |
| 166 | 3 | 4 | 7 | 5 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 130 | 3 |
| 132 | 3 |
| 134 | 13 |
| 136 | 3 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 127 | 2 |
| 129 | 11 |
| 131 | 8 |
| 133 | 4 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 127 | 2 |
| 129 | 11 |
| 131 | 8 |
| 133 | 4 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 129 | 1 |
| 131 | 14 |
| 133 | 5 |
| 135 | 3 |
| 137 | 1 |

* maximum hatch possible in each replicate is 25

Appendix VIII (Cont.)

6.2 HOURS DAILY

| Time in hours | no. hatching * | | | |
|---|---|---|---|---|
| 178 | 0 | 4 | 0 | 0 |
| 180 | 15 | 13 | 18 | 9 |
| 182 | 4 | 1 | 1 | 11 |
| 184 | 3 | 5 | 1 | 0 |
| 186 | 0 | 0 | 1 | 0 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 129 | 1 |
| 131 | 9 |
| 133 | 3 |
| 135 | 10 |

8.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 192 | 4 | 1 | 2 | 3 |
| 194 | 11 | 14 | 10 | 13 |
| 196 | 4 | 3 | 5 | 7 |
| 198 | 4 | 4 | 4 | 0 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 129 | 1 |
| 131 | 14 |
| 133 | 5 |
| 135 | 4 |

10.4 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 230 | 0 | 0 | 0 | 1 |
| 232 | 7 | 8 | 8 | 10 |
| 234 | 7 | 7 | 10 | 8 |
| 236 | 9 | 9 | 7 | 4 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 134 | 1 |
| 136 | 17 |
| 138 | 7 |

* maximum hatch possible in each replicate is 25

Appendix VIII (Cont.)

13.5 HOURS DAILY

| Time in hours | no. hatching* | | | |
|---|---|---|---|---|
| 286 | 3 | 3 | 1 | 3 |
| 288 | 3 | 3 | 2 | 2 |
| 290 | 11 | 13 | 13 | 11 |
| 292 | 2 | 2 | 2 | 3 |
| 294 | 4 | 2 | 2 | 1 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 132 | 1 |
| 134 | 17 |
| 136 | 7 |

17.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 452 | 8 | 7 | 7 | 8 |
| 454 | 6 | 8 | 9 | 9 |
| 456 | 10 | 8 | 8 | 6 |
| 458 | 0 | 1 | 1 | 2 |

CHECK

| Time in hours | no. hatching |
|---|---|
| 128 | 1 |
| 130 | 3 |
| 132 | 4 |
| 134 | 10 |
| 136 | 5 |

* maximum hatch possible in each replicate is 25

Appendix VIII (Cont.)

DAILY EXPOSURE TO 5 ° C. OF 24 HOUR OLD EGGS

CHECK 1-2.2 Hrs.

| Time in hours | no. hatching* |
|---|---|
| 129 | 3 |
| 131 | 3 |
| 133 | 2 |
| 135 | 1 |

CHECK 2.9-3.7 Hrs.

| Time in hours | no. hatching |
|---|---|
| 129 | 2 |
| 131 | 5 |
| 133 | 1 |
| 135 | 1 |

CHECK 4.8-10.4 Hrs.

| Time in hours | no. hatching |
|---|---|
| 133 | 2 |
| 135 | 4 |
| 137 | 2 |

CHECK 13.5-22.5 Hrs.

| Time in hours | no. hatching |
|---|---|
| 133 | 1 |
| 135 | 4 |
| 137 | 3 |

1.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 133 | 5 | 2 | 3 | 1 |
| 135 | 3 | 5 | 4 | 2 |
| 137 | 1 | 2 | 2 | 5 |
| 139 | 1 | 1 | 1 | 2 |

1.3 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 135 | 1 | 0 | 0 | 0 |
| 137 | 1 | 4 | 1 | 1 |
| 139 | 6 | 6 | 8 | 8 |

1.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 137 | 1 | 1 | 1 | 0 |
| 139 | 4 | 1 | 5 | 4 |
| 141 | 4 | 5 | 1 | 3 |
| 143 | 1 | 2 | 2 | 2 |

2.2 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 139 | 3 | 3 | 2 | 2 |
| 141 | 2 | 3 | 4 | 4 |
| 143 | 3 | 4 | 4 | 2 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

2.9 HOURS DAILY

| Time in hours | no. hatching* | | | |
|---|---|---|---|---|
| 142 | 0 | 0 | 0 | 1 |
| 144 | 3 | 4 | 1 | 1 |
| 146 | 5 | 5 | 4 | 4 |
| 148 | 1 | 1 | 4 | 2 |

4.8 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 158 | 1 | 1 | 0 | 1 |
| 160 | 4 | 2 | 2 | 2 |
| 162 | 3 | 5 | 6 | 4 |

8.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 180 | 0 | 1 | 0 | 1 |
| 182 | 2 | 3 | 3 | 1 |
| 184 | 3 | 2 | 3 | 5 |
| 186 | 4 | 2 | 1 | 0 |

13.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 256 | 1 | 1 | 0 | 2 |
| 258 | 3 | 4 | 6 | 3 |
| 260 | 4 | 1 | 4 | 3 |

3.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 148 | 4 | 5 | 2 | 3 |
| 150 | 2 | 1 | 3 | 4 |
| 152 | 3 | 2 | 3 | 2 |
| 154 | 1 | 1 | 0 | 0 |

6.2 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 169 | 0 | 0 | 1 | 0 |
| 171 | 3 | 1 | 2 | 1 |
| 173 | 5 | 2 | 2 | 2 |
| 175 | 1 | 3 | 3 | 3 |

10.4 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 229 | 3 | 4 | 3 | 2 |
| 231 | 2 | 0 | 2 | 2 |
| 233 | 1 | 3 | 2 | 2 |

17.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 384 | 1 | 1 | 0 | 1 |
| 386 | 2 | 2 | 3 | 4 |
| 388 | 6 | 5 | 4 | 4 |
| 390 | 0 | 1 | 2 | 0 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

DAILY EXPOSURE TO 5 ° C. OF 48 HOUR OLD EGGS

CHECK 1-4.8 Hrs.

| Time in hours | no. hatching* |
|---|---|
| 130 | 2 |
| 132 | 4 |
| 134 | 3 |

CHECK 6.5-33.5 Hrs.

| Time in hours | no. hatching |
|---|---|
| 131 | 2 |
| 133 | 4 |
| 135 | 2 |
| 137 | 1 |

1.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 136 | 1 | 2 | 0 | 3 |
| 138 | 5 | 3 | 4 | 2 |
| 140 | 2 | 2 | 4 | 4 |
| 142 | 1 | 2 | 2 | 1 |

1.3 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 137 | 0 | 0 | 1 | 0 |
| 139 | 3 | 3 | 4 | 4 |
| 141 | 4 | 4 | 4 | 4 |
| 143 | 2 | 2 | 0 | 1 |

1.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 140 | 2 | 2 | 1 | 3 |
| 142 | 3 | 3 | 2 | 2 |
| 144 | 4 | 4 | 3 | 3 |
| 146 | 1 | 0 | 4 | 1 |

2.2 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 142 | 1 | 1 | 1 | 1 |
| 144 | 4 | 3 | 3 | 4 |
| 146 | 2 | 4 | 4 | 4 |
| 148 | 2 | 0 | 0 | 0 |

2.9 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 146 | 0 | 2 | 0 | 1 |
| 148 | 1 | 1 | 1 | 2 |
| 150 | 6 | 6 | 6 | 3 |
| 152 | 2 | 0 | 1 | 4 |

3.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 152 | 1 | 1 | 2 | 1 |
| 154 | 3 | 3 | 4 | 2 |
| 156 | 3 | 3 | 2 | 5 |
| 158 | 2 | 2 | 2 | 1 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

4.8 HOURS DAILY

| Time in hours | no. hatching* | | | |
|---|---|---|---|---|
| 156 | 0 | 0 | 0 | 1 |
| 158 | 1 | 3 | 3 | 3 |
| 160 | 5 | 4 | 4 | 5 |
| 162 | 2 | 2 | 2 | 0 |

6.2 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 162 | 0 | 2 | 0 | 1 |
| 164 | 1 | 2 | 1 | 2 |
| 166 | 5 | 4 | 5 | 2 |
| 168 | 4 | 1 | 3 | 3 |

8.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 173 | 0 | 1 | 1 | 1 |
| 175 | 3 | 3 | 3 | 5 |
| 177 | 5 | 4 | 5 | 4 |
| 179 | 1 | 1 | 0 | 0 |

10.4 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 192 | 2 | 2 | 2 | 1 |
| 194 | 2 | 4 | 3 | 2 |
| 196 | 4 | 3 | 3 | 6 |
| 198 | 1 | 0 | 1 | 0 |

13.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 234 | 3 | 0 | 2 | 3 |
| 236 | 2 | 3 | 4 | 3 |
| 238 | 3 | 4 | 3 | 3 |
| 240 | 1 | 1 | 0 | 0 |

17.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 336 | 1 | 0 | 1 | 0 |
| 338 | 3 | 1 | 4 | 2 |
| 340 | 4 | 5 | 4 | 5 |
| 342 | 1 | 2 | 0 | 1 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

DAILY EXPOSURE TO 5 ° C. OF 96 HOUR OLD EGGS

CHECK 1-2.2 hrs.

| Time in hours | no. hatching* |
|---|---|
| 130 | 2 |
| 132 | 2 |
| 134 | 4 |
| 136 | 1 |

CHECK 2.9 hrs.

| Time in hours | no. hatching |
|---|---|
| 130 | 4 |
| 132 | 2 |
| 134 | 2 |
| 136 | 1 |

CHECK 8.0 HOURS

| Time in hours | no. hatching |
|---|---|
| 131 | 3 |
| 133 | 2 |
| 135 | 4 |

CHECK (remaining reps)

| Time in hours | no. hatching |
|---|---|
| 129 | 3 |
| 131 | 4 |
| 133 | 2 |

1.0 HOURS DAILY

| Time in hours | no. hatching* |
|---|---|
| 132 | 4 3 4 3 |
| 134 | 2 4 2 3 |
| 136 | 2 2 4 3 |

1.3 HOURS DAILY

| Time in hours | no. hatching |
|---|---|
| 133 | 1 0 1 2 |
| 135 | 5 4 4 4 |
| 137 | 4 3 3 1 |
| 139 | 0 2 1 2 |

1.7 HOURS DAILY

| Time in hours | no. hatching |
|---|---|
| 134 | 0 2 1 1 |
| 136 | 4 4 2 4 |
| 138 | 3 3 5 4 |
| 140 | 2 0 1 1 |

2.2 HOURS DAILY

| Time in hours | no. hatching |
|---|---|
| 134 | 1 0 0 0 |
| 136 | 3 3 3 5 |
| 138 | 4 4 5 4 |
| 140 | 1 1 0 0 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

2.9 HOURS DAILY

| Time in hours | no. hatching* | | | |
|---|---|---|---|---|
| 136 | 2 | 1 | 2 | 1 |
| 138 | 3 | 2 | 2 | 4 |
| 140 | 3 | 4 | 5 | 3 |
| 142 | 0 | 1 | 0 | 0 |

4.8 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 139 | 2 | 2 | 2 | 3 |
| 141 | 4 | 3 | 2 | 2 |
| 143 | 1 | 3 | 3 | 4 |
| 145 | 2 | 2 | 2 | 1 |

8.0 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 153 | 1 | 0 | 0 | 0 |
| 155 | 4 | 3 | 3 | 4 |
| 157 | 3 | 2 | 2 | 2 |
| 159 | 1 | 3 | 4 | 2 |

13.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 182 | 1 | 2 | 1 | 2 |
| 184 | 2 | 3 | 1 | 2 |
| 186 | 4 | 3 | 6 | 5 |
| 188 | 2 | 0 | 0 | 0 |

3.7 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 138 | 3 | 2 | 2 | 1 |
| 140 | 2 | 5 | 5 | 3 |
| 142 | 4 | 2 | 2 | 4 |
| 144 | 0 | 1 | 1 | 0 |

6.2 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 142 | 0 | 2 | 3 | 0 |
| 144 | 3 | 3 | 1 | 4 |
| 146 | 4 | 3 | 2 | 3 |
| 148 | 2 | 2 | 2 | 1 |

10.4 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 160 | 3 | 3 | 2 | 2 |
| 162 | 4 | 5 | 4 | 3 |
| 164 | 2 | 1 | 2 | 2 |
| 166 | 0 | 0 | 1 | 2 |

17.5 HOURS DAILY

| Time in hours | no. hatching | | | |
|---|---|---|---|---|
| 216 | 0 | 1 | 0 | 1 |
| 218 | 3 | 3 | 3 | 4 |
| 220 | 3 | 4 | 4 | 3 |
| 222 | 2 | 1 | 2 | 1 |

* maximum hatch possible in each replicate is 10

Appendix VIII (Cont.)

22.5 HOURS DAILY

| Time in hours | no. hatching* | | | |
|---|---|---|---|---|
| 432 | 1 | 1 | 1 | 1 |
| 434 | 2 | 4 | 3 | 2 |
| 436 | 4 | 4 | 2 | 5 |
| 438 | 2 | 0 | 2 | 1 |
| 440 | 0 | 0 | 1 | 0 |

* maximum hatch possible in each replicate is 10